

ANALYTICAL SENSITIVITY OF TSH ASSAYS BY ELISA AND ELFA

Dr.K.Pramila¹, Dr.Poonguzhali Gopinath², Dr.K.Menaka Shanthi², Dr.M.Divya³Professor¹, Asst.Professor², Final Year Post graduate³ - Department of Biochemistry
ICH & HC, Madras Medical College, Chennai.

ABSTRACT

INTRODUCTION : The technological advances from radioimmunoassay to immunometric assays have greatly enhanced the sensitivity of TSH assays over the last three decades and has firmly established TSH as the single most reliable Thyroid function test in assessing the Thyroid hormone status of an individual.

AIMS AND OBJECTIVE : The objective of this current study is to compare the performance of TSH assay based on Enzyme Linked Fluorescent Assay (VIDAS system) with Enzyme Linked Immunosorbant assay technology (Accubind) and also to compare the analytical sensitivity of the TSH assay by the above two technologies.

MATERIALS AND METHODS : Thyroid stimulating hormone levels have been assayed in the serum of 105 patients by both ELISA and ELFA so as to analyze the performance of the above two technologies. The term analytical sensitivity refers to a minimal concentration of TSH that can be detected by an assay with greater confidence.

RESULTS : There exists statistically significant difference between assays by ELISA and ELFA in Euthyroid ($p < 0.001^{**}$) and Hypothyroid samples ($p < 0.05$). The precision CV for Hyperthyroid samples by ELISA range from 7.7% to 39.4% whereas for ELFA the precision CV ranges from 3.4% to 14.5% thus exhibiting the greater analytical sensitivity for ELFA.

CONCLUSION : TSH assay by ELFA has broader functional range, higher analytical sensitivity and also higher specificity compared to TSH assay by ELISA technology. The actual purpose of this study is to fully characterize the analytical performance of the two assays, to understand the capability and limitations of each test so as to ensure that they are fit for routine use.

KEY WORDS : ELISA, ELFA, Thyroid stimulating hormone, analytical sensitivity

INTRODUCTION

Thyroid stimulating hormone is the heterodimeric glycoprotein (α and β) secreted from the anterior pituitary gland. The assay of thyrotropin is useful in detecting thyroid related disorders. The technological advances from radioimmunoassay to immunometric assays have greatly enhanced the sensitivity of TSH assays over the last three decades and has firmly established TSH as the single most reliable Thyroid function test in assessing the Thyroid hormone status of an individual^{1,2}. The

sensitivity of TSH assay has been further enhanced by utilization of chemiluminescent and fluorescent signals that has additional advantage of easier to automate^{3,4}.

There exists a physiological log/linear inverse relationship between circulating TSH and FT4 concentrations⁵. In fact, altered TSH level is the earliest abnormality to be detected in a person with abnormal Thyroid function⁶. TSH assay is the excellent screening test for the detection of Thyroid hormone status in an individual, but not without disadvantages. In case of

Address for Correspondence :

Dr.K.PRAMILA, M.D., Professor of Biochemistry, ICH & HC, Madras Medical College, Chennai.
Email : dr.k.pramila@gmail.com Phone no: 9444144099

hypothyroidism due to hypothalamic Thyrotropin releasing hormone deficiency the TSH assays with current immunometric methodologies is within normal limits. This is because TRH from Hypothalamus is responsible for glycosylation of TSH more than release of TSH from pituitary, so in TRH deficiency TSH is non-glycosylated. This non-glycosylated TSH is biologically inactive but retains immunoreactivity, so detected by current TSH immunometric assays as normal TSH levels even though the hormone is biologically inactive⁷.

There are various immunometric assays depending on the signals used :

- Immunoradiometric assay (IRMA) – It uses radio-isotope signals
- Immunoenzymometric assay (IEMA)- It uses enzyme signals
- Immunofluorometric assay (IFMA)- It uses fluorophores as signals
- Immunochemiluminometric assay (ICMA)-It uses chemiluminescent molecules as signals
- Immunobioluminometric assay (IBMA) – It uses bioluminescent molecules as signals

The current study compares the Immunoenzymometric assay ELISA with Immunofluorometric assay ELFA. Enzyme linked immunosorbent assay has been proved to be a useful test for the detection of TSH status of an individual. Enzyme linked fluorescent assay is newer, simple, reliable as well as sensitive method for the assay of TSH. Both are immunometric assays, in ELISA the enzyme hydrolyze the substrate to yield a colored product and in ELFA the enzyme acts on the substrate to produce a fluorescent product.

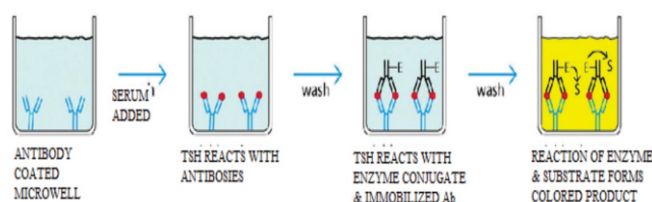
MATERIALS AND METHODS

Blood samples were collected from 105 children visiting Endocrinology OPD, Institute of child Health, Egmore. Newborn babies and children with acute illness were excluded from the study. Whole blood was collected and subjected to centrifugation and the separated serum is aliquoted in two fresh microcentrifuge tubes– one for ELISA and other for ELFA TSH Assays.

PRINCIPLE OF ELISA:

Monoclonal biotinylated antibodies directed against TSH are coated on the microwell plates. TSH in serum is allowed to react simultaneously with two antibodies, being sandwiched between solid phase and enzyme linked antibodies (Fig 1). This sandwich complex is immobilized to the well through high affinity streptavidin- biotin interaction. After incubation at room temperature for an hour, wells are washed to remove excess antibodies. Addition of substrate results in the formation of colored product. The enzyme and substrate is allowed to react for 15 minutes by incubation in room temperature. Stop solution is added at the end of 15 minutes and the intensity of the color developed is measured by ELISA reader set at 450nm.

FIG 1: SANDWICH ELISA



PRINCIPLE OF ELFA:

The principle of ELFA is same as that of ELISA which utilize immunoassay sandwich methodology except that instead of enzyme substrate reaction forming a colored product, a final fluorescent product is formed by reaction of the enzyme with substrate which is then detected. The advantage of ELFA methodology is that it can easily be automated.

REFERENCE INTERVAL FOR TSH:

The setting of reference interval for TSH is critical for the diagnosis of mild hypo or hyperthyroidism. Current guidelines recommend that “TSH reference intervals should be established from the 95 percent confidence limits of the log-transformed values of at least 120 rigorously screened normal euthyroid volunteers, who should not have any detectable thyroid antibodies, TPOAb or TgAb (measured by sensitive immunoassay), do not have personal or family history of thyroid dysfunction and have no visible or palpable goiter, and are not taking any medications”.¹ Recent studies have suggested that TSH level increases in

elderly and that a mild TSH elevation in elderly individuals may even convey a survival benefit⁸⁻¹⁰. Also the TSH population reference range for adults will not apply to neonates and children. Serum TSH values are generally higher in neonates and children.¹¹⁻¹³. All these studies point out the importance of establishing age specific reference interval for thyroid¹⁴. The reference interval for TSH given by CALIPER study for the children in the age group of six months to 14 years is 0.7 to 4.17mIU/L¹¹. Although Single nucleotide polymorphism in genes responsible for Thyroid hormone synthetic pathway (like PDE8B and TSH receptor genes) may account for the euthyroid outliers and account for skewing of established reference intervals^{15,16} the utility of population based reference range is very limited due to narrow inter individual variability¹⁷. According to Marwaha et al¹⁸ >95% of Indian school children have TSH level between 5.28-8.40µIU/mL and the mean serum TSH level is 1.83-3.58µIU/mL. The reference range provided by the manufacturer for ELISA kit is 0.39-6.16µIU/mL and by ELFA it is 0.25-5.0µIU/mL. Serum TSH exhibits a diurnal variation, that peaks between midnight to 04:00 hours¹⁹⁻²¹. But all the blood samples are collected during out-patient timing of 7:30 AM to 10:00 AM, hence diurnal variation of TSH, practically has no impact on this study. Current guidelines states that determination of TSH levels in serum should be the first line screening test for the detection of sub-clinical and overt hyper or hypothyroidism²². Hence utilization of highly sensitive test for serum TSH determination is the need of the time which is the purpose of this test.

RESULTS

Out of 105 individuals, ELISA identifies 66 children to be Euthyroid, 12 as Hyperthyroid and 27 children are identified as being Hypothyroid (Table 1). ELFA identifies only 63 as Euthyroid, 10 children as Hyperthyroid and 32 children were classified as Hypothyroid (Table 2).

The mean of Thyroid status of the individuals with ELISA and ELFA were compared by unpaired student't' test and p-Value was computed (Table 3). There is statically significant difference between ELISA and ELFA in Euthyroid ($p < 0.001^{**}$) and Hypothyroid ($p < 0.05^*$) individuals. There is insignificant difference in mean value between hyperthyroid individuals in the study.

ELISA has 27 as Hypothyroid and ELFA identifies 32 as Hypothyroid. All the 27 patients have been compared (Fig 2). ELISA identifies 12 as Hyperthyroid and ELFA identifies 10 as Hyperthyroid. All the 10 patients have been compared (Fig 3).

10 Hyperthyroid samples were selected and each sample is run twice in ELISA and ELFA for a period of 3 days (Between run and between day variability is determined) (Table 4). 10 Hypothyroid samples were selected and each sample is run twice in ELISA and ELFA for a period of 3 days (Between run and between day variability is determined) (Table 5).

Table1: ELISA: EU/HYPO/HYPER THYROID INDIVIDUALS

THYROID STATUS	N (105)	MEAN (µIU/mL)	STANDARD DEVIATION (µIU/mL)
EUTHYROID	66	3.25	±1.57
HYPERTHYROID	12	0.24	±0.07
HYPOTHYROID	27	27.8	±24.8

Table 2 ELFA: EU/HYPO/HYPER THYROID INDIVIDUALS

THYROID STATUS	N (105)	MEAN (µIU/mL)	STANDARD DEVIATION (µIU/mL)
EUTHYROID	63	2.36	±1.3
HYPERTHYROID	10	0.49	±0.07
HYPOTHYROID	32	42.1	±25.4

Table 3: COMPARISON OF THYROID STATUS BY ELISA AND ELFA :

MEAN	ELISA (µIU/mL)	ELFA (µIU/mL)	P-value
EUTHYROID	3.25±1.57	2.36±1.3	*0.001 **HS
HYPOTHYROID	27.8±24.8	42.1±25.4	*0.05 ~S
HYPERTHYROID	0.24±0.07	0.49±0.07	NS

FIG 2: Comparison between ELISA AND ELFA methodology for Hypothyroid subjects:

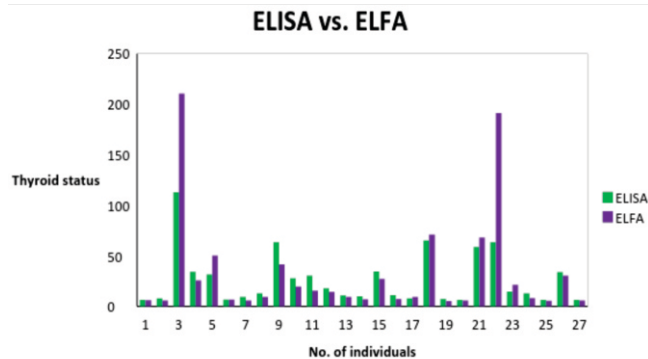


FIG 3: Comparison between ELISA AND ELFA methodology for Hyperthyroid subjects:

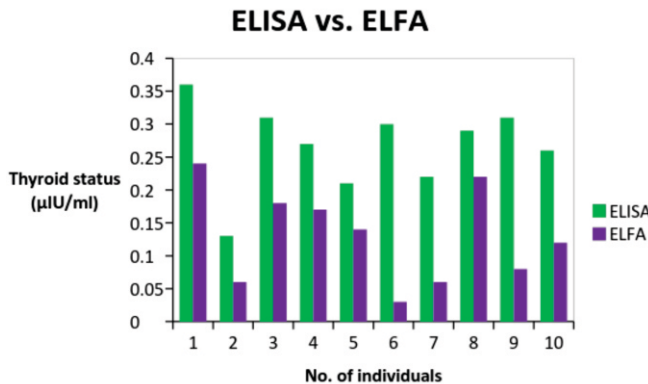


Table 4: Precision accuracy of ELISA and ELFA in Hyperthyroid:

SAMPLE NO.		1	2	3	4	5	6	7	8	9	10
ELISA	MEAN (μIU/ml)	0.41	0.32	0.29	0.14	0.26	0.31	0.38	0.17	0.3	0.42
	SD(μIU/ml)	0.07	0.04	0.06	0.05	0.07	0.05	0.03	0.05	0.05	0.05
	CV(%)	17.52	13.81	20.6	39.4	26.01	18.05	7.7	28.4	16.2	14.02
ELFA	MEAN (μIU/ml)	0.23	0.25	0.32	0.05	0.25	0.37	0.36	0.07	0.2	0.42
	SD (μIU/ml)	0.01	0.01	0.01	0.007	0.02	0.01	0.01	0.01	0.01	0.01
	CV(%)	6.1	4.11	5.4	14.5	6.91	3.6	3.5	18.07	4.1	3.5

Table 5: Precision accuracy of ELISA and ELFA in Hypothyroid :

SAMPLE NO.		1	2	3	4	5	6	7	8	9	10
ELISA	MEAN (μIU/ml)	14.3	43.83	132	255.3	76.5	74.5	151.83	21.5	49.3	40.1
	SD(μIU/ml)	1.86	7.7	13.5	30.5	6.2	6.94	11.82	4.84	3.82	4.44
	CV(%)	12.99	17.5	10.2	11.9	8.15	9.32	7.78	22.5	7.7	11.06
ELFA	MEAN (μIU/ml)	15.3	48.6	137.6	282.5	86.5	77.8	153.33	28	54.3	45.6
	SD(μIU/ml)	1.36	1.75	2.16	10.09	3.59	2.48	3.20	2.52	2.06	1.6
	CV(%)	8.9	3.59	1.56	3.57	4.15	3.19	2.08	9.22	3.8	3.57

DISCUSSION:

The radioimmunoassay for TSH measurement in the initial era has limit of detection of 1 μIU/ml. Introduction of immunometric assays have improved the sensitivity by 10 fold, hence samples with TSH levels as low as 0.1 μIU/ml can easily be detected²³. The immunometric assays are inherently more sensitive and has additional advantage that they can easily be automated²⁴. However, the limit of detection is not sufficient for TSH and even lower levels are to be detected to differentiate between sub-clinical and overt hyperthyroidism. Hence it is need of time to utilize highly sensitive assay for thyroid.

The assay of TSH by ELISA identifies 66 individuals as Euthyroid, but ELFA picked up only 63 persons to be Euthyroid, there is statistically significant difference ($p < 0.001^{**}$) between ELISA and ELFA for Euthyroid individuals. ELISA identifies 27 persons as hypothyroid but ELFA detects 32 persons as hypothyroid. The precision CV of ELISA for hypothyroid samples ranges from 7.7% to 22.5%, whereas precision CV of ELFA for hypothyroid samples ranges from 1.56% to 9.22%. There is statistically significant difference as computed by student- t test between ELISA and ELFA for the hypothyroid samples ($p < 0.05^{*}$). There is insignificant difference between ELISA and ELFA for the hyperthyroid samples, possibly due to lower sample size and the degree of significance is not valid. The precision CV of ELISA for hyperthyroid samples is 7.7% to 39.4% whereas for ELFA it is 3.4% to 14.5%.

The Nomenclature Committee of the American Thyroid Association has recommended that Analytical detection limit of TSH assay should be determined on the basis of Lower end inter-assay precision characteristics. The committee reported that precision co-efficient of variation at the lower reporting limit should be 10-15%, but no worse than 20%²⁵. The present study has got better lower end precision CV for ELISA in the range of 0.2-0.3 μIU/ml whereas by ELFA technology there is better lower end precision CV in the range of 0.05-0.06 μIU/ml. So assay of TSH by fluorescent

signals has better detection limit compared to the assay based on utilization of enzymatic signals.

Currently there is no available reference measurement procedure (RMP) for TSH assay. So all the current available assays refer traceability to World Health Organization (WHO) TSH International Reference Preparation 80/558, but all of them do not produce comparable results. This is probably because of the fact that the WHO standard is not commutable, which means that the levels of TSH are not within meaningful limits by different measurement procedures. This characteristic can be attributed to many factors, possibly due to alteration of either the sample matrix or the measurand during production of a reference material. This leads to reference materials reacting differently to different measurement procedures. Hence utilization of non-commutable standards for calibration traceability is not feasible²⁶. The IFCC's Committee for Standardization of Thyroid Function Tests (C-STFT) proposes "harmonization of TSH using a new measurement standard: a panel of native materials with values assigned by the all-procedure trimmed mean as the surrogate RMP".

CONCLUSION :

The utilization of fluorescent signals for assay of TSH is inherently more sensitive than utilization of enzyme signals. The added advantage of Fluorescent technology is that they can easily be automated. The assay of TSH based on fluorescent technology provided by VIDAS system utilizes Solid phase Receptacle (SPR) that acts both as solid phase and pipetting device. All the required reagents are pre-packed in the sealed reagent strips and all the steps are carried out automatically by the instrument. In conclusion, we found that TSH assay by VIDAS system (based on ELFA technology) is highly automated, reliable and also highly efficient for the assay of TSH compared to ELISA. ELFA technology can detect TSH levels as low as 0.05 μ IU/ml. Assay of TSH based on fluorescent signal has higher precision and analytical sensitivity as well as broader functional range compared to assay of TSH by enzymatic signals.

REFERENCES :

1. Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Henry JF, et al. Laboratory medicine practice guidelines. Laboratory support for the diagnosis and monitoring of thyroid disease. *Thyroid*. 2003;13(1):3-126.
2. Baskin HJ, Cobin RH, Duick DS, Gharib H, Guttler RB, Kaplan MM, et al. American Association of Clinical Endocrinologists medical guidelines for clinical practice for the evaluation and treatment of hyperthyroidism and hypothyroidism. *Endocr Pract*. 2002;8(6):457-469.
3. Fiore M, Mitchell J, Doan T, Nelson R, Winter G, Grandone C, et al. The Abbott IMx automated benchtop immunochemistry analyzer system. *Clin Chem*. 1988;34(9):1726-1732.
4. McCapra F, Watmore D, Sumun F, Patel A, Beheshti I, Ramakrishnan K et al. Luminescent labels for immunoassay--from concept to practice. *J Biolumin Chemilumin*. 1989;4(1):51-58.
5. Meier CA, Maisey MN, Lowry A, Muller J, Smith MA. Interindividual differences in the pituitary-thyroid axis influence the interpretation of thyroid function tests. *Clin Endocrinol (Oxf)*. 1993;39(1):101-107.
6. Meikle AW, Stringham JD, Woodward MG, Nelson JC. Hereditary and environmental influences on the variation of thyroid hormones in normal male twins. *J Clin Endocrinol Metab*. 1988;66(3):588-592.
7. Benhadi N, Fliers E, Visser TJ, Reitsma JB, Wiersinga WM. Pilot study on the assessment of the setpoint of the hypothalamus-pituitary-thyroid axis in healthy volunteers. *Eur J Endocrinol*. 2010;162(2):323-329.
8. Atzmon G, Barzilai N, Surks MI, Gabriely I. Genetic predisposition to elevated serum thyrotropin is associated with exceptional longevity. *J Clin Endocrinol Metab*. 2009;94(12):4768-4775.
9. Gussekloo J, van Exel E, de Craen AJ, Meinders AE, Frolich M, Westendorp RG. Thyroid status,

- disability and cognitive function, and survival in old age. *JAMA*. 2004;292(21):2591-2599.
10. Mariotti S. Thyroid function and aging: do serum 3,5,3'-triiodothyronine and thyroid-stimulating hormone concentrations give the Janus response? *J Clin Endocrinol Metab*. 2005;90(12):6735-6737.
 11. Chan MK, Seiden-Long I, Aytekin M, Quinn F, Ravalico T, Ambruster D et al. Canadian Laboratory Initiative on Pediatric Reference Interval Database (CALIPER): pediatric reference intervals for an integrated clinical chemistry and immunoassay analyzer, Abbott ARCHITECT ci8200. *Clin Biochem*. 2009;42(9):885-891.
 12. Kapelari K, Kirchlechner C, Hogler W, Schweitzer K, Virgolini I, Moncayo R. Pediatric reference intervals for thyroid hormone levels from birth to adulthood: a retrospective study. *BMC Endocr Disord*. 2008;8:15.
 13. Zurakowski D, Di Canzio J, Majzoub JA. Pediatric reference intervals for serum thyroxine, triiodothyronine, thyrotropin, and free thyroxine. *Clin Chem*. 1999;45(7):1087-1091.
 14. Kahapola-Arachchige KM, Hadlow N, Wardrop R, Lim EM, Walsh JP. Age-specific TSH reference ranges have minimal impact on the diagnosis of thyroid dysfunction. *Clin Endocrinol (Oxf)*. 2012;77(5):773-779.
 15. Arnaud-Lopez L, Usala G, Ceresini G, Mitchell BD, Pilia MG, Piras MG, et al. Phosphodiesterase 8B gene variants are associated with serum TSH levels and thyroid function. *Am J Hum Genet*. 2008;82(6):1270-1280.
 16. Peeters RP, van der Deure WM, Visser TJ. Genetic variation in thyroid hormone pathway genes; polymorphisms in the TSH receptor and the iodothyronine deiodinases. *Eur J Endocrinol*. 2006;155(5):655-662.
 17. van de Ven AC, Netea-Maier RT, Medici M, Sweep FC, Ross HA, Hofman A et al. Underestimation of effect of thyroid function parameters on morbidity and mortality due to intra-individual variation. *J Clin Endocrinol Metab*. 2011;96(12):E2014-2017.
 18. Marwaha RK, Tandon N, Desai A, Kanwar R, Grewal K, Aggarwal R et al. Reference range of thyroid hormones in normal Indian school-age children. *Clin Endocrinol (Oxf)*. 2008;68(3):369-374.
 19. Brabant G, Prank K, Hoang-Vu C, Hesch RD, von zur Muhlen A. Hypothalamic regulation of pulsatile thyrotropin secretion. *J Clin Endocrinol Metab*. 1991;72(1):145-150.
 20. Evans PJ, Weeks I, Jones MK, Woodhead JS, Scanlon MF. The circadian variation of thyrotrophin in patients with primary thyroidal disease. *Clin Endocrinol (Oxf)*. 1986;24(4):343-348.
 21. Weeke J, Gundersen HJ. Circadian and 30 minutes variations in serum TSH and thyroid hormones in normal subjects. *Acta Endocrinol (Copenh)*. 1978;89(4):659-672.
 22. Garber JR, Cobin RH, Gharib H, Hennessey JV, Klein I, Mechanick JI et al. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. *Endocr Pract*. 2012;18(6):988-1028.
 23. Seth J, Kellett HA, Caldwell G, Sweeting VM, Beckett GJ, Gow SM, et al. A sensitive immunoradiometric assay for serum thyroid stimulating hormone: a replacement for the thyrotrophin releasing hormone test? *Br Med J (Clin Res Ed)*. 1984;289(6455):1334-1336.
 24. Self CH, Cook DB. Advances in immunoassay technology. *Curr Opin Biotechnol*. 1996;7(1):60-65.
 25. Hay ID, Bayer MF, Kaplan MM, Klee GG, Larsen PR, Spencer CA. American Thyroid Association assessment of current free thyroid hormone and thyrotropin measurements and guidelines for future clinical assays. The Committee on Nomenclature of the American Thyroid Association. *Clin Chem*. 1991;37(11):2002-2008.
 26. Vesper HW, Miller WG, Myers GL. Reference materials and commutability. *Clin Biochem Rev*. 2007;28(4):139-147.

Received on 04/01/2016, Modified on 06/04/2016, Accepted on 24/06/2016