



## COMPARATIVE ASSESSMENT OF QUALITY OF IMMUNORADIOMETRIC ASSAY (IRMA) AND CHEMILUMINESCENCE IMMUNOMETRIC ASSAY (CHEIMA) FOR ESTIMATION OF THYROID STIMULATING HORMONE (TSH)

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Biological substances like hormones, vitamins and enzymes are found in minute quantities in blood. Their estimation requires very sensitive and specific methods. The most modern method for estimation of thyroid stimulating hormone in serum is non-isotopic enzyme enhanced chemiluminescence immunometric method. In our laboratory immunoradiometric assay is in routine for the last many years. Recently interest has grown to establish non-isotopic techniques in laboratories of PAEC. However, the main requirement to adopt the new procedures is to compare their results, cost and other benefits with the existing method. Immunoassay laboratory of MINAR, therefore, conducted a study to compare the two methods. A total of 173 (males: 34 females: 139 age: between 1 and 65 years) cases of clinically confirmed thyroid status were included in the study. Serum samples of these cases were analyzed by two methods and results were compared by plotting precision profiles, correlation plots and calculating sensitivities and specificities of the methods. As the results in all the samples were not normally distributed Wilcoxon rank sum test was applied to compare the analytical results of two methods. The comparison shows that the results obtained in two methods are not completely similar ( $p=0.0003293$ ), although analysis of samples in groups shows that some similarity exists between the results of hypo and hyperthyroid patients ( $p<=0.156$  and  $p<=0.6138$ ). This shows that results obtained in these two methods could sometimes disagree in final diagnosis. Although TSH-CHEIMA is analytically more sensitive than TSH-IRMA the clinical sensitivities and specificities of two methods are not significantly different. TSH-CHEIMA test completes in almost 2 hours whereas TSH-IRMA takes about 6 hours to complete. Comparison of costs shows that TSH-CHEIMA is almost 5 times more expensive than TSH-IRMA. We conclude that the two methods could sometimes disagree but the two techniques have almost same clinical efficacy (clinical usefulness). The clinical sensitivities and specificities are similar and TSH-IRMA is in no way inferior to the non-isotopic method. It is, therefore, not reasonable to abandon a good technique only for relatively speedy results.

**Keywords :** IRMA, CHEIMA, Immunoradiometric, TSH-IRMA,

### 1. Introduction

Biochemical changes in human body are very important for proper functioning of various organs. The most important changes are those related to growth and physiology of the body. The catalytic compounds which regulate these changes are hormones, enzymes and vitamins [1-2]. The quantities of these compounds in blood are so small that we need special methods to estimate them. Very sensitive and specific methods were developed in the years 1960 to 1970. These methods employed reaction between diluted antibody with antigen (hormone) in serum and a radiolabelled antigen. The method was called radioimmunoassay [3]. The technique is still very popular and is available in almost all biochemical laboratories of the world. The technique has undergone several changes and is now-a-days an automated technique. Scientists have also been trying to develop more sensitive and specific

methods to replace this conventional method. For example an immunoassay method with improved precision and specificity employing concentrated amount of antibody was developed. This method is called immunoradiometric assay or IRMA. This technique has now almost dominated RIA.

Due to their radioactive nature IRMA and RIA techniques have been criticized by many (although the radiation hazard is very little with these techniques when we compare them with non-isotopic methods which employ the use of many carcinogenic chemicals). Many alternate immunoassays were therefore developed in the last quarter of 20<sup>th</sup> century [4]. Among these chemiluminescence immunoassays are very popular. Although these methods are advanced and attractive to laboratory personnel they have produced some problems in laboratories of developing countries. The main question about these methods is whether they are superior to existing methods or not. The main drawback in these methods is that

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the antigen is conjugated with a big molecule at some non specific site and it is assumed that it will not affect the specific properties of the molecule. In our opinion this assumption is not correct. In IRMA and RIA we label the molecules with I-125 which does not significantly affect the biochemical nature of the labeled compounds.

This article describes the economical and technical problems in two methods (in the context of measurement of TSH in blood) and after technical comparison of these we have tried to develop a strategy to decide about selection or rejection of these methods. Other laboratories could also follow such strategies in adapting new procedures. In our opinion it is expedient that a laboratory should adopt those procedures that are relatively cheaper, good in quality, simple and quick.

Thyroid stimulating hormone is produced in pituitary and controls the amounts of T<sub>3</sub> and T<sub>4</sub> produced in the thyroid. These hormones are very important for proper metabolism and growth of human body. Their deficiency or excess causes diseases like hypo and hyperthyroidism. TSH is present in human blood in concentrations from 0.5 to 5  $\mu$ IU/l (roughly 70 to 350 pg/ml). Such small quantities require very sensitive methods for their estimation. Efforts to develop such methods started in the last century in fifties and two American scientists Berson and Yalow [3] introduced a technique called radioimmunoassay or RIA. Amounts in the range of 1/10000000 g/l were measured in this method. Here an antigen (analyte or substance to be measured) is labeled with an isotope (I<sup>125</sup>). This labeled antigen is called tracer. This tracer and it's analogue in blood compete for a limited number of binding sites on the specific antibody. This gives antigen antibody complex. The amount of antigen in blood is calculated from the amount of radioactivity in antigen-antibody complex. The technique has undergone many changes since its introduction and is now applied worldwide. The use of computer has solved many of its problems related to smooth performance. The changes in this technique has not changed its very nature but removed its deficiencies and now it is almost completely automated.

The scientists have also been engaged in developing other techniques similar to RIA but based on different principles, for example immunoradiometric technique introduced by Nick Hales [5]. This technique was a step forward in the field of immunoassay and has been very popular

during last two decades. The method established at our laboratory for the measurement of TSH is Third Generation TSH Immunoradiometric TSH-assay (TSH-IRMA). The technique is claimed to have more sensitivity, specificity and relatively wide working range compared to old RIA procedures [6-9]. However, every technique has its own limitations which must be kept in mind when applying it for diagnosis. Different commercial companies have different claims for applicability of such methods. Such claims must be independently and scientifically tested and verified before accepting a methodology for final application, otherwise this could cause increase in error of laboratory measurement and wastage of capital.

Radioimmunoassay technique at our institute was introduced in late seventies of last century and is still functioning. The procedures have been changing with time but are still dominating in our laboratories. New methods have however emerged in last years based on the use of non-isotopic labels. Many commercial companies claim increased sensitivity, specificity and accuracy for these methods [10-12]. Enzyme amplified chemiluminescence immunometric technique have gained much popularity among these [13, 14]. We, therefore, decided to evaluate this relatively modern method and compare it with our routine method. We were successful to install an automated system at our institute. Manufactured in America, the machine is called immulite. The immulite technique was introduced by an American company DPC(Diagnostic product company) few years back and the technique is available in most modern laboratories of the world.

The main objective of this study was to suggest a strategy to immunoassay laboratories to improve their performance by evaluating the cost, quality and speed of analysis of the existing and new procedures. We believe that decisions about selection/rejection of existing/new methodologies should be based on sound statistical data obtained after proper experimental studies. This is very important to achieve correct clinical diagnosis and to save our national economy.

## 2. Materials and Methods

### 2.1. Patients

A total of 173 clinically confirmed patients (Males: 34, Females: 139, Age: 1-65 years) were selected for study. These patients were referred to MINAR after provisional diagnosis by their treating physicians. Here they and their previous records were re-examined by our nuclear physicians and

their final diagnosis was based on clinical history, thyroid scans and thyroid related hormone tests (T<sub>3</sub>-RIA, T<sub>4</sub>-RIA and TSH-IRMA).

### 2.2. Blood samples

About 5 cc blood of each patient was collected by venepuncture. The blood was allowed to clot for half an hour and serum was separated from it and stored at 4°C until use.

### 2.3. Laboratory tests

Each serum was tested twice, first using TSH-IRMA and then by chemiluminescence immunometric assays (TSH-CHEIMA). For TSH-IRMA kits of Immunotech Company France were used [15] whereas for TSH-CHEIMA immulite kits (DPC USA) were employed [16]. The basic

principles of these methods are described below:

### 2.4. Chemiluminescence immunometric assays (TSH-CHEIMA)

This technique was introduced by Babson in 1991 [17]. The most modern form of this technique is two site solid phase sequential chemiluminescent immunometric assay technique or CHEIMA. An automatic immunoassay analyzer or immulite is used to perform these assays. A specific antibody coated on a plastic bead is kept in a proprietary test unit (Fig. 1) which acts as a reaction vessel. Other reactants like alkaline phosphatase labeled second antibody (Ab-2) and chemiluminescent enzyme substrate are also added into it. Briefly 50 µl serum sample, antibody on plastic bead and alkaline phosphatase labeled second antibody are

The heart of IMMULITE is the proprietary Test Unit which an assay-specific antibody-coated bead, serves as the reaction vessel for all sample processing. Spinning the Test Unit at high speed efficiently expels fluid into the integral sump chamber. The tube design allows for multiple discrete washes within seconds, ensuring excellent separation of unbound material for highly sensitive assays. IMMULITE's unique wash technique produces very low and consistent nonspecific binding, which is critical for assay performance.

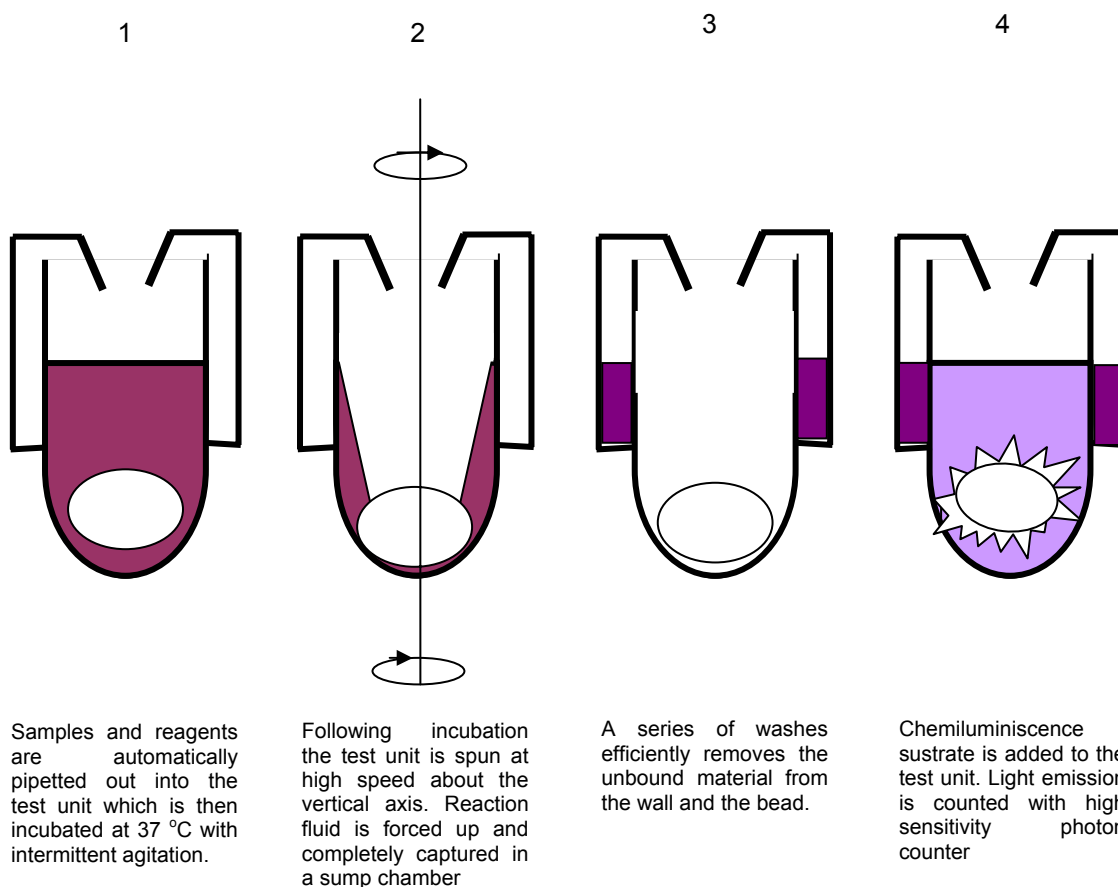


Figure 1. Principle of chemiluminescence immunometric assay.

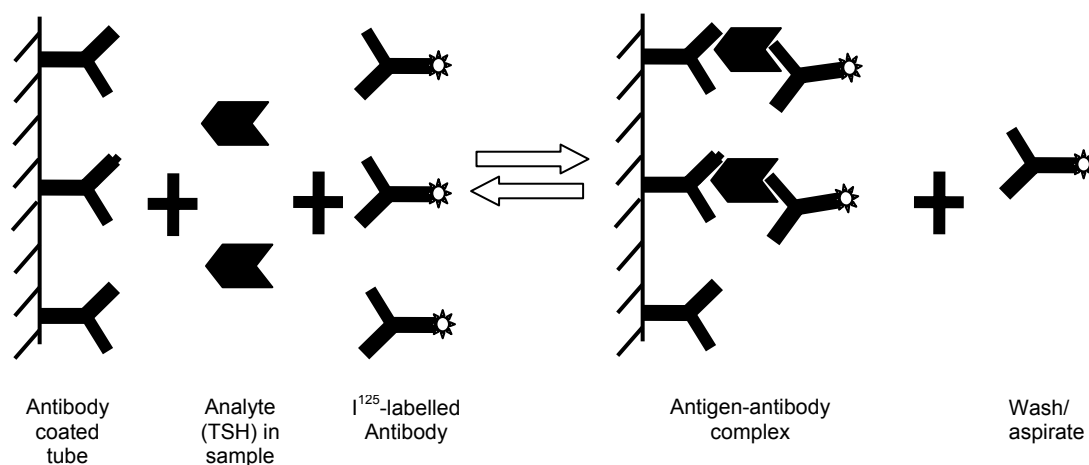


Figure 2. Principle of Immunoradiometric assay.

kept for incubation at 37°C for 1 hour. The unreacted components are then very quickly removed from the reaction well by spinning the test unit vertically along its own axis and the bead is washed repeatedly. All the liquid waste material is transferred to waste chamber of test unit during this spinning process and there is no undesired substrate or enzyme reagent with the bead. Chemiluminescence substrate is then added. Now quantity of photo emission is determined by a photomultiplier tube in a dark chamber. A photo sensor connected with this dark chamber sends signals to a computer which analyzes these signals and calculates the concentration of hormone in blood and sends all the analytical data to the printer to print the results report. It takes about two hours to complete this process.

#### 2.5. Two site immunoradiometric TSH assay technique

Here serum sample is added to an antibody coated plastic tube alongwith  $I^{125}$ -labelled monoclonal antibody. TSH in serum is attached to the walls of the tube and labeled monoclonal antibody then binds to TSH making an antibody-antigen-antibody sandwich. The tube walls are now washed with wash buffer to separate undesired reactants and products. The tubes are then counted in a gamma counter to determine the amount of radioactivity in the sandwich. This radioactivity is analyzed by a computer and TSH levels in blood samples are calculated. The principle of the technique is shown in Fig. 2.

#### 2.6. Product data supplied by the manufactures

The brochures supplied with immunoassay kits (immunotech and immulite) showed an assay sensitivity of 0.004 uIU/ml for TSH-Immulite and 0.025 uIU/ml for TSH-IRMA respectively. Both the methods claimed to have acceptable specificity and did not show any cross reactivity with molecules like LH, FSH, GH and prolactin. The working range for Immulite-TSH was upto 75  $\mu$ IU/ml whereas for Immunotech –TSH it was 0.025-50  $\mu$ IU/ml. Recovery experiments showed recovery between 93 and 106% for immunotech assay whereas immulite-assay showed a recovery of 92-119%. The linearity of dilution was also appropriate in these two methods. Immulite assay maintains 92% linearity at very low dilution (1:64) whereas immunotech assay maintains linearity between 92% and 106%. Thus comparison of technical data supplied by the manufacturers of these kits shows that Immulite-TSH is relatively more sensitive.

#### 2.7. Data and statistical analysis

As stated earlier each serum sample was analyzed twice first by TSH-IRMA (immunotech) and then by TSH-CHEIMA (immulite). Results (counts) obtained in immunotech kits were analyzed using a gamma counter (Stratec, Germany) attached with a computer and result calculation was done by a microprocessor in the computer. Data from Immulite kits was analyzed by immulite system. Comparative analysis of results was done by comparing quality control sample results, precision

Table 1. Mean TSH levels in euthyroid, hypothyroid and hyperthyroid subjects

No	Nature of sample	Number	TSH Level (µIU/ml; mean ±SD) IRMA	TSH Level (µIU/ml; mean ±SD) CHEIMA	P (Wilcoxon rank sum test)	Correlation Coefficients CHIEMA versus IRMA
1.	All subjects	173(M:34 F:139)	2.81±9.43	4.14±9.89	p = 0.0003293	r=0.84 A=-0.54 B=0.799
2.	Euthyroid	122(M:22 F:100)	1.98±2.13	1.09±1.55	p<= 1.345e-08	r=0.83 A=-0.114 B=0.599
3.	Hyperthyroid	27(M:4 F:23)	1.54±2.17	2.36±3.37	p <= 0.156	r=0.999 A=0.02 B=0.0.645
4	Hypothyroid	24(M: 6 F:28)	16.15±23.95	21.28±22.69	p <= 0.6138	r=0.78 A=-1.43 B=0.83
5	QC1 (Low)	n=10	0.318±0.040 (12.7%)	0.202±0.015 (7.4%)		
6	QC2 (Medium)	n=10	2.125±0.102 (4.8%)	1.615±0.018 (1.1%)		
7	QC3 (High)	n=10	20.47±0.553 (2.7%)	15.55±0.055 (0.32%)		

Expected values of pooled QCs: QC1: Mean±SD= 0.26±0.05 µIU/l range: 0.15-0.367 QC2: Mean ± SD = 2.2 ± 0.296 µIU/l range:1.61-2.796 QC3: Mean±SD = 24.5±2.386 µIU/l range: 19.75-29.9. The values are averaged on 400 samples of each pool.

Table 2. Observed clinical sensitivities and specificities of the techniques

Samples	Clinical Sensitivity IRMA	Clinical Sensitivity CHEIMA	Clinical Specificity IRMA	Clinical specificity CHEIMA	Analysis of Significance
Euthyroid	-----	-----	90.4%	86.3%	*NS
Hyperthyroid	81.8%	84.4%	-----	-----	NS
Hypothyroid	85.2%	79.2%	-----	-----	NS
Overall	83.05%	81.67%	-----	-----	NS

\*NS-not significant

profile, regression analysis [18-20]. Significance values were derived by applying Wilcoxon rank sum test because the data in our normal and diseased population was not normally distributed. Regression analysis was done to check the linearity of results between two methods. A regression or correlation coefficient of less than 0.95 was taken as negative for linear correlation.

### 3. Results and Discussion

Chemiluminescence immunometric assay (TSH-CHEIMA) technique is very attractive for almost all laboratories of the world because of its complete automation that saves labour. Its use is increasing day by day not only in health but also in veterinary sciences [21]. However in developing countries such techniques produce economic constraints especially when we already have a cheaper alternate of good quality but we need to compare our existing techniques with these new developments to see whether we are losing enhanced quality (which the commercial suppliers claim) or not. Therefore, the qualitative and

quantitative data we obtained in this study is discussed as below:

TSH levels found in euthyroid, hyperthyroid, hypothyroid and quality control pools are given in Tables 1 and 2 respectively. The results of regression analysis are displayed in Figures 3 to 6.

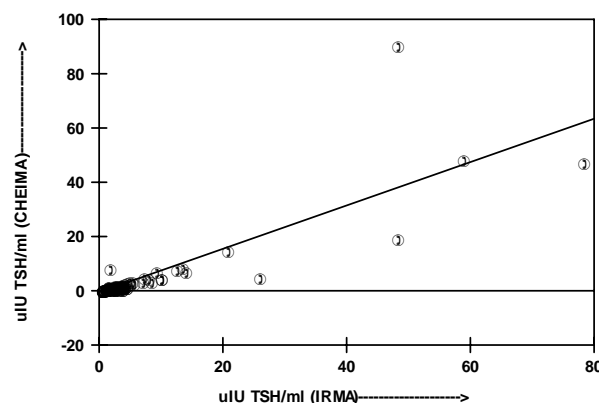


Figure 3. Correlation between IRMA and CHEIMA (All Subjects).

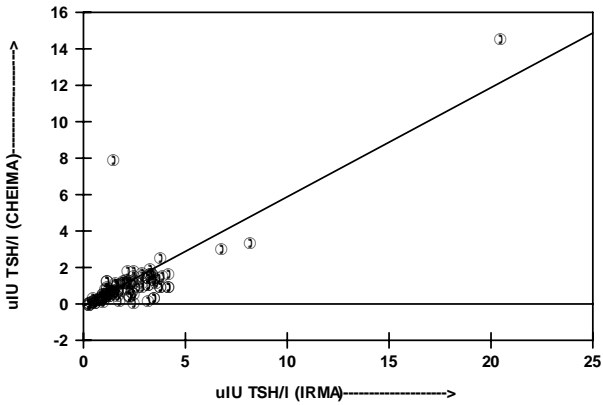


Figure 4. Correlation between IRMA and CHEIMA (Euthyroid Subjects)

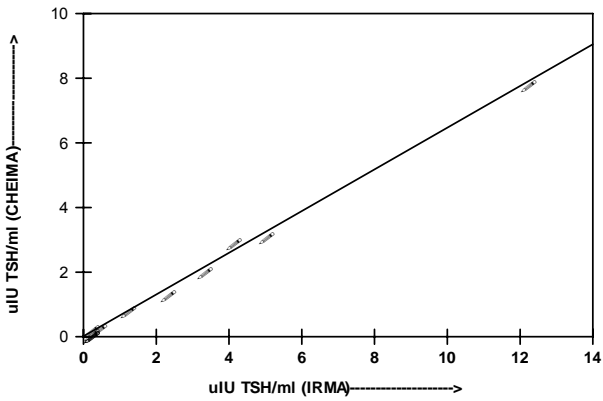


Figure 5. Correlation between IRMA and CHEIMA (Hyperthyroid Subjects).

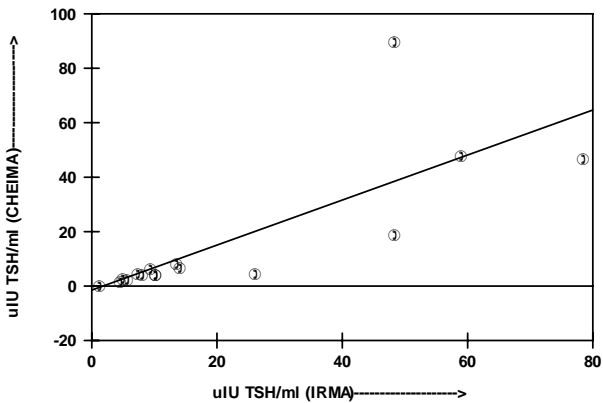


Figure 6. Correlation between IRMA and CHEIMA (Hypothyroid Subjects).

The precision profiles obtained in two assays are shown in figures 7 and 8. The comparison of precision profiles is shown in Fig. 9. The mean TSH levels observed in our patients in these two techniques were:  $2.82 \pm 9.43 \mu\text{IU/ml}$  (IRMA) and  $4.14 \pm 9.89 \mu\text{IU/ml}$  (CHEIMA) respectively. Statistical

Working ranges (uIU TSH/ml): TSH-CHEIMA intraassay  
MINAR: 0.09-->30  
TSH-CHEIMA intraassay DPC: 0.16-->20  
TSH-CHEIMA interassay DPC: 1-->20

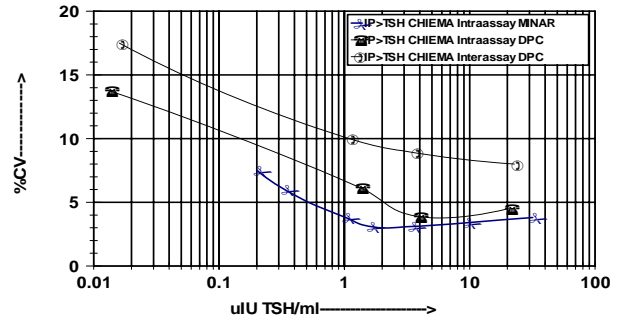


Figure 7. Precision Profile-TSH CHEIMA.

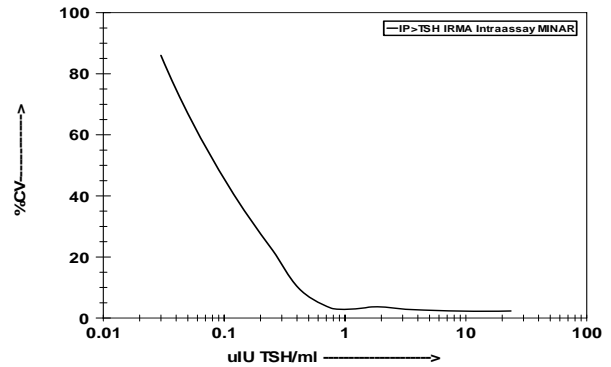


Figure 8. Precision Profile-IRMA (Working range: <0.3 to >25).

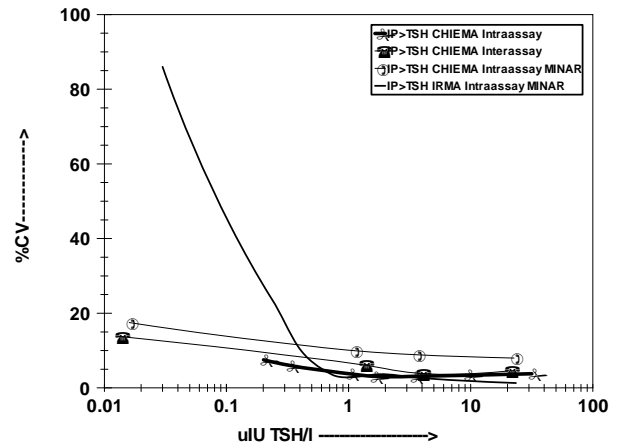


Figure 9. Precision Profiles-CHEIMA.

analysis (Wilcoxon test) shows lack of similarity in these two methods ( $p=0.0003293$ ). Regression analysis shows a weak linear relationship in two

methods ( $r=0.84$ ). However analysis of results in other groups shows resemblance in the results. In hypothyroid group, there is a linear relation between the results of two techniques ( $r=0.999$ ) and the values are also not significantly different ( $p \leq 0.156$ ). It is worth mentioning here that that in both the methods there are some patients with normal TSH levels although they are not disease free. It is evident that both the procedures can not detect hyperthyroid patients with 100% efficiency. Therefore, both the procedures have almost equal efficacy for hyperthyroid subjects. Almost same picture is seen in hypothyroid patients i.e., results are not significantly different ( $p=0.6138$ ) but with poor linearity among them ( $r=0.78$ ). There is significant difference among TSH levels of euthyroid subjects ( $p \leq 0.000000134$ ). The linear relationship is however poor as well. So there may be some diagnostic problems in these subjects.

The quality control sample results show that the values are within expected limits in two methods. The comparison of clinical sensitivity in two methods shows that the clinical sensitivity is almost same in two procedures. The clinical specificities of TSH-IRMA (90%) and TSH-CHEIMA (86.3%) are also not significantly different.

Comparison of precision profiles shows that the immulite TSH-assay done at our laboratory is more precise compared to intra and interassay precision of immulite assays performed by DPC (Fig. 7). TSH-IRMA is also very precise (Fig. 8) but TSH-CHEIMA (Fig. 9) is relatively more precise at lower levels (this reflects the high analytical sensitivity of the technique). An overall picture shows almost similar precision of two methods. The prices provided to MINAR for the year 2007-2008 show following custom free rates: TSH-CHEIMA, 100 test=150 U\$ and TSH-IRMA, 100 test= 28U\$. Therefore TSH-CHEIMA is almost 5 times more expensive than TSH-IRMA. There is almost 35% increase in rates in current fiscal year.

#### 4. Conclusion

In the light of above discussion it is concluded that although the two methods sometimes disagree in diagnosis because of relatively high analytical sensitivity of TSH-CHEIMA, the clinical sensitivities and specificities of the two techniques are not significantly different. Therefore, in our opinion two techniques have almost same clinical efficacy (clinical usefulness) and it is not reasonable to abandon a good method merely on the basis of better automation and speedy results.

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