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# Production and Characterization of Immunoradiometric Technique for Measuring of Thyroglobulin in Human Serum

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ABSTRACT

**The development of the thyroglobulin immunoradiometric assay (Tg-IRMA) using solid phase coated plates is the primary goal of this work. Three components were prepared and characterized for the Tg-IRMA method. The first component was polyclonal antibody of thyroglobulin (anti-Tg), that was generate via immunized three Balb/C mice against highly purified Tg antigen. The vaccination schedule was followed, consisting of the initial vaccination and three booster injections. The second component was radioiodinated both of Tg antigen and Tg monoclonal antibody (Tg-MoAb) with radioactive iodin-125 (Na $^{125}$I) to prepare two radioactive tracers, first one was $^{125}$I-Tg* radioactive tracer used for characterized the produced anti-Tg polyclonal and second one was $^{125}$I–Tg-MoAb* radioactive tracer to use in established solid phase coated-plate Tg-IRMA technique. Additionally, set of Tg standards with different concentration was prepared and evaluated with commercial Tg-IRMA Dia-Source kits. The optimization and validation tests were studies to obtained accurate, precis and valid local prepared system. Finally, locally produced solid phase coated-plate Tg-IRMA may be very useful in the in-vitro evaluation of thyroglobulin in human serum to diagnose and treat disorders of hyperthyroidism and hypothyroidism, as well as in following the progress of treatment for thyroid cancer and recognize recurrence. In general, preparing the immunoradiometric system is considered one of the most important diagnostic radiological applications in the practical medical field**

## INTRODUCTION

Thyroglobulin (Tg) is a large glycoprotein that has a molecular weight of within 660 kDa. In thyroid follicular cells, Tg is responsible for the synthesis of T3 and T4 [1]. Thyroxine (T4) and triiodothyronine (T3) are released into the bloodstream as a result of TSH's stimulation of endocytosis at the apical membrane and proteolysis of Tg, which controls Tg in thyroid follicular cells. Thyroglobulin is in all vertebrates the main precursor to thyroid hormones, which are produced when thyroglobulin's tyrosine residues are combined with iodine and the protein is subsequently cleaved. In each thyroglobulin molecule, there are around 100-120 tyrosine residues. However, only a limited amount (20) of these residues are capable of being iodinated by thyroperoxidase within the follicular colloid. About 10 molecules of thyroid hormone are formed by Tg molecules [2, 3]. Elevated levels of Serum Tg can be observed in patients with various thyroid conditions including goiter, benign thyroid nodules, thyroid adenomas, multinodular goiters, thyrotoxicosis, and during the toxic phase of thyroiditis [3].

Thyroglobulin assays are commonly utilized as a means of monitoring patients diagnosed with differentiated thyroid carcinoma, acting as a reliable tumor marker. The detection of an increased thyroglobulin level is a clear indicator of a potential recurrence of papillary or follicular thyroid cancer. In other words, an increase in thyroglobulin levels in the bloodstream may indicate the growth and/or spread of thyroid cancer cells. Detection of thyroglobulin antibodies may indicate immune system-mediated damage to the thyroid gland [4]. One way to ensure proper monitoring for potential recurrence of thyroid

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cancer is by measuring thyroglobulin antibody (Tg-Ab) levels after treatment [5]. Serum tests have revealed the presence of Tg-Ab in patients with autoimmune thyroid disorders, such as Hashimoto's thyroiditis and Graves' disease. Also, serum Tg measurements can be utilized in infants to assess the underlying cause of congenital hypothyroidism. Thyroglobulin is measured by competitive and immunometric assays. With this background the measurement of thyroglobulin in human sera is increasingly being used all over the world, as a significant tool in diagnosis of thyroid dysfunctions and possible thyroid carcinoma [4, 5].

Radioimmunoassay's (RIAs) are highly sensitive micro-analytical techniques that are widely regarded as the most effective means of determining extremely low concentrations of various substances with biological and medical significance. In fact, no other analytical method comes close to achieving the low detection limits of RIA-related techniques. The widespread applicability, ease of use, and cost-effectiveness of RIA have played a crucial role in advancing medical research over the last fifty years [6].

Immunoassays that are non-competitive are the immunoradiometric assays (IRMAs) [7]. For assessing the majority of hormones, particularly peptides and proteins, IRMAs are the preferred technique [8]. Saturating concentrations of two or more antisera that identify non-competitive epitopes in the analyte are used in IRMAs, as opposed to RIAs. In order to bind every analyte in an unknown sample, IRMAs need to be immobilized to a solid phase matrix of capture antibodies in sufficient excess [9].

A capture antibody is initially passively adsorbed or covalently bonded to the surface of a solid phase in a typical non-competitive assay for an antigen. Both types of antibodies can be used in non-competitive assays (IRMA) for capture and labeling. A capture antibody is initially passively adsorbed or covalently bonded to the surface of a solid phase in a typical non-competitive assay for an antigen [10].

This study amid to develop solid phase coated plate thyroglobulin immunoradiometric assay (Tg–IRMA) technique, the following essential components will be prepare and characterize to achieve a valid and precise technique. The included components consist of an anti-Tg polyclonal, $^{125}$I-Tg* and $^{125}$I-Tg-MoAb* radioactive tracers and Tg standards.

## MATERIALS AND METHODS

### Materials

Highly purified thyroglobulin antigen (T-6830), Goat anti-mouse IgG (M-8019), monoclonal anti-thyroglobulin antibody produced in mouse clone 2H11 (SAB4701013), Polyethylene glycol-8000 (P-4463), Freund's adjuvant complete (F-5881) and incomplete (F-5506) were bought from Sigma–Aldrich. Radioactive iodine ($Na^{125}I$, 10 mCi/100 µl) pH (7-11) was bought from center of Izotop (Budapest, Hungary), commercial kit of Tg-IRMA (Dia-Source). Polyvinyl plates for coating obtained from Greiner, Germany. The additional chemical ingredients were all of analytical grade and got from reliable suppliers.

### Animals

Three male of Balb /C mice were used as a host animals, each mouse weighing about 30 g. Mice were got from the animal housing in the labeled compound department, Hot Labs Center-Egypt. Mice were maintained in sterile conditions with a well-balanced food and unlimited access to water.

### Methods

#### *Polyclonal antibodies of thyroglobulin*

The generation of thyroglobulin polyclonal antibody (anti-Tg) against thyroglobulin antigen (Tg) was undertaken via the immunization schedule reported in previous studies [11, 12] by immunized the mice with doses of Tg antigen as a follow:

Highly purified Tg antigen (immunogen) was dissolved in normal saline (1 mg Tg /1 ml) as stock. For primary immunization, each mouse was received 50 µg Tg antigen in 50 µl sodium chloride (0.9 %) emulsified with 50 µl complete Freund's adjuvants (CFA) at ratio (1:1). The emulsified immunogen was injected intraperitoneal at 5 sites (20 µl in each site). About the booster doses, each mouse was received 25 µg of Tg antigen in 50 µl sodium chloride (0.9 %) emulsified with 50 µl incomplete Freund's adjuvant (IFA) at ratio (1:1). Emulsification will be performed using Hamilton double-hub syringes connected to each other with narrow metallic tubing [13]. Four immunization injections were administered, one primary and three booster injections, at two weeks intervals [14, 15].

All mice were bled through cut the end of tail or the corner of eye by fine capillary tube and serum was separated from the blood without any additives and was

kept under – 20 °C till testing. Titre of dilution and displacement percentage of the obtained antisera were determined using RIA technique [15]. The anti-serum (anti-Tg) was diluted in assay buffer (0.05 M phosphate, pH 7.4, containing 0.15 M NaCl) to determine the titre of the antibody. The dilution ranged from 1:50 to 1:109,350 fold [16].

***Characterization of Anti-Tg polyclonal:***

Titer and displacement were used to assess anti-Tg polyclonal. The titer of the harvested antisera for Tg (as a first Ab) was determined by the double antibody RIA technique using goat anti-mouse IgG (as a second antibody), non-immune goat serum (NGS), and PEG-8000 as a separating agent.

The following procedure was used for evaluated the dilution titre of antibody: 100 µl of Tg antisera at various dilutions (1/50, 1/150, 1/450, 1/1,350, 1/4,050, 1/12,150, 1/36,450, 1/109,350) added to 100 µl of Tg standards (0.0 or 250 ng/ml), 100 µl of $^{125}$I-Tg* radioactive tracer counted~ 30,000 cpm with 200 µl assay buffer, and incubating for a period of three hours at room temperature.

The following separation formula was added to each test tube in order to separate the bound and free fraction: 100 µl of diluted goat anti-mouse IgG (1:10), 100 µl of diluted NGS (1:100), and 0.5 ml of polyethylene - glycol (PEG-8000) at dilution 4%. Assay tubes were centrifuged at 4000 rpm for 15 minutes at 4 °C after being incubated for 15 minutes at room temperature. The precipitates contained the bound fraction that was measured using a gamma counter. The binding % for bleeding for every mouse was determined using a logit-log graph [16, 17].

The following equation was applied to calculate the highest displacement percentage between the maximum binding percentage ($B_0$%, 0.0 ng/ml) and minimum binding percentage ($B_H$%, 250 ng/ml) for each individual collected antisera for each mouse:

$$Displacement\ (D\%) = B_O\% - B_H\ \%/B_O\%.$$

This was done by using two of the zero and high Tg standards (0.0 and 250 ng/ml) to construct different antibody dilution curves.

After producing anti-Tg with the appropriate titre and displacement, ammonium sulphate precipitation procedure was used to partially purify the antiserum. This was followed by dialysis using 0.02 M phosphate buffer [15, 18]. Highly purified anti-Tg polyclonal (IgG-Tg) was created by completely purification the partially purified anti-Tg polyclonal using the protein-A column affinity purification method after the dialysis process [15]. Concentration of immunoglobulin-Tg (IgG-Tg) in purify anti-Tg was determined by Ultra Violet spectrophotometer at absorbance 280 nm. The following formula was used for calculating the IgG-Tg concertation: $^{0.1\%}E_{280} = 1.4$

Until use, the purified anti-Tg polyclonal was kept at – 20 °C.

***Tg Standards:***

The preparation of the Tg stock standard was dissolving 1 mg of pure human Tg antigen in 1 ml of 0.05 M phosphate buffer saline (pH 7.4), which included 5 ml of NaCl (3 M), 0.1 g of sodium azide, and 0.1 g of bovine serum albumin (BSA) per 100 ml [19]**.** Working Tg standards ranged from (0.5, 1.0, 5.0, 20, 100, 250 ng/ml) were prepared and estimated values using commercial Tg-IRMA kit (Dia-Source) to assure their validity.

***Radioiodination of Thyroglobulin***

***$^{125}$I-Tg* and $^{125}$I-MoAb-Tg* radioactive tracers:***

The preparation of radioactive $^{125}$I-Tg* or $^{125}$I-MoAb-Tg* tracers were carried out by Iodegen oxidation method according to Fraker and Speck [20]. $^{125}$I-Tg* radioactive tracer was used for assessment of the prepared anti-Tg polyclonal using RIA technique and $^{125}$I-MoAb-Tg* radioactive tracer used for measuring Tg in serum by IRMA technique.

***$^{125}$I-Tg* radioactive tracer for RIA***

Eppendorf vial was coated by Iodogen (100 µg in $CHCl_3$), the vial was leaved to dray over night at room temperature. After that, 10 µl of phosphate buffer (0.5 M, pH, 7.4) was added to Eppendorf vial coated by Iodogen followed by 10 µl Tg antigen (10 µg Tg in phosphate buffer 0.05 M, pH, 7.4), then mixed vial with gentleness. The reaction was initiated by addition of 5 µl (500 µCi) of radioactive iodine (Na $^{125}$I) to vail. After 15 minutes at room temperature, the reaction was ended by transferring the reaction mixture to another vial. Labeled reaction mixture was separated and purified by using Sephadex G-75 column. The collected fractions for $^{125}$I-Tg* radioactive tracer were counted by Radioisotope Dose Calibrator. The elution profile was constructed by drawing a count against fractional numbers, which also yielded the radioactivity % and free iodide for $^{125}$I- Tg* radioactive tracer. The fractions of $^{125}$I-Tg* radioactive tracer produced was kept at 4 °C till use.

### *$^{125}$I-MoAb-Tg* radioactive tracer for IRMA*

The Eppendorf vial was treated with 100 µg Iodogen in CHCl3 for coating and the vial was left to dray over night at room temperature. Next, 10 µl of 0.5 M phosphate buffer with a pH of 7.4 was added to an Eppendorf vial coated with Iodogen. This was followed by the addition of 10 µl of MoAb-Tg (10 µg in 0.05 M phosphate buffer with a pH of 7.4) to the vial. The vial was then gently mixed. The initiation of the reaction was achieved by adding 5 µl (500 µCi) of radioactive iodine (Na $^{125}$I) to a vial. The reaction was terminated by transferring the reaction mixture to another vial after being left at room temperature for 15 minutes. The labeled reaction mixture was subjected to separation and purification using a Sephadex G-75 column. The Radioisotope Dose Calibrator was used to count the collected fractions of $^{125}$I-MoAb-Tg* radioactive tracer. The elution profile of the $^{125}$I-MoAb-Tg* radioactive tracer was created by plotting the count against fractional numbers. This also provided the radioactivity and free iodide percentage. The obtained ($^{125}$I-MoAb-Tg*) radioactive tracer was stored at 4 $^{o}$C until needed.

### Preparation of coated plate Tg-IRMA

#### *The process of coating*

The method of coating were carried out as described by Pierce and Klinman [21] as a follow: polyvinyl plates (96 - well) were coated with 100 µl (12 µg/ well) of the purified anti-Tg (IgG-Tg) in phosphate buffer saline (0.02 M, pH 7.4) and incubate over night at room temperature. The plates were washed three times with phosphate buffer saline (0.02 M, pH 7.4) which contain 0.1% $NaN_3$ and the plates were blocked by 1% BSA. After incubating at 25 °C for the overnight, the plates were decanted without being washed. The coated plates were kept at 4 $^{o}$C until use

#### *The assay design and optimization of Tg-IRMA*

The optimum conditions of solid phase coated-plates Tg-IRMA technique for determining Tg were done by study some parameter such as: volume of sample, time of incubation and process of washing. The study was performed using a standard or sample volume (25, 50, or 100 µl), 100 µl of produced radioactive tracer ($^{125}$I-MoAb-Tg) counted at about 140,000 cpm, an incubation time at (1, 2, 3, and 24 hours), and 200 µl of three washing volume (once, twice, and third). The gamma counter was used to count the plates, and log-logit graph paper was used to calculate the data.

#### *Validity tests of Tg-IRMA:*

The validity of coated-plate Tg-IRMA was ensured through investigations on many parameters, including specificity, sensitivity and precision, accuracy and technique comparability.

## RESULTS AND DISCUSSION

To produce anti-Tg polyclonal antibodies, three Balb/C mice were immunized with Tg antigen as a follow: 100 µl emulsion containing (50 µg Tg in 50 µl saline / 50 µl CFA) / mouse for the primary immunization, and 100 µl emulsion containing (25 µg Tg in 50 µl saline / 50 µl IFA) / mouse to provide each booster. All mice had an effective immunological response, as indicated by the final findings. The dilution titre and displacement of the antiserum were determined based on the present bind of a fixed amount (100 µl) of $^{125}$I-Tg* radioactive tracer through various dilutions ranging from 1/50 to 1/109,350 of collected antisera Tg for every mouse using zero and high (0 and 250 ng/ml) standards, as illustrated in (Fig. 1). According to the findings, at dilution titre 1/12,150, mouse 1 (M1) and mouse 2 (M2) both provided the highest displacement values, 69.5% and 68.7%, respectively. Furthermore, at dilution titre 1/4,050, mouse 3 (M3) produced the highest displacement value of 72.2%.

Based on the acquired data, all mice (M1, M2, and M2) had the higher displacement percentage and good immunological response of Tg polyclonal antibody between zero and high Tg standards (0.0 and 250 ng/ml). To assess Tg levels in human serum, a solid phase-coated plate (Tg-IRMA) was created using the pool of anti-Tg from all mice (M1, M2, and M3). This pool was purified before being used as a basic component of the diagnostic kit.

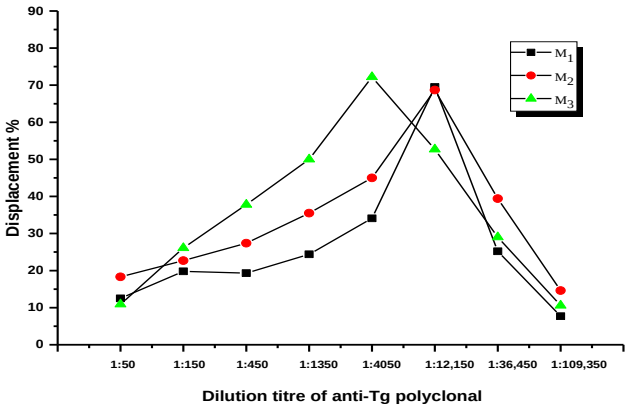


**Fig. (1): Dilution titre and displacement % of anti-Tg polyclonal for all mice ($M_1$, $M_2$, $M_3$).**

The obtained antiserum of Tg from all mice ($M_1$, $M_2$ and $M_3$) was pooled and it was subjected to complete purification via the following steps, first one antiserum purified using ammonium sulfate precipitation method, second one complete purification using protein A-column. The protein content of the purified IgG-Tg was quantified using a UV spectrophotometer at a wavelength of 280 nm. The content was determined using the following equation:

$$^{0.1\%}E_{280} = 1.4$$

Comparing the obtained pure IgG-Tg to the IgG reference, the concentration of IgG-Tg equaled 4.9 mg/ml. The purified of anti-Tg polyclonal were used in coating process in solid phase coated plate Tg-IRMA technique.

In this study, highly pure human Tg antigen (1 mg/ml) in phosphate buffer was used to prepare Tg standards ranging from (0.5 - 250 ng/ml). Set of the prepared Tg standards were assessed with DIA Source Tg-IRMA kit. As illustrated in Table (1) the findings for locally produced Tg standards that were obtained gave a good recovery between expected and observed values ranged from 97% to 104%.

**Table (1): Evaluation of the Tg standards produced locally using the Tg-IRMA kit (Dia Source).**

| Standards | Expected value (ng/ml) | Observed value (ng/ml) | Recovery % (O/E) |
|---|---|---|---|
| **1** | 0.5 | 0.51 | 102 |
| **2** | 1.0 | 1.04 | **104** |
| **3** | 5.0 | 4.9 | 98 |
| **4** | 10 | 10.3 | 103 |
| **5** | 20 | 19.4 | **97** |
| **6** | 100 | 98.5 | 98.5 |
| **7** | 250 | 244.5 | 97.8 |

Using Iodogen as an oxidizing agent, radio-iodinated Tg ($^{125}$I-Tg* tracer) and ($^{125}$I- MoAb-Tg* tracer) were performed. By plotting activity (µCi) against fraction number, the gel chromatography elution profile for each of $^{125}$I-Tg* and $^{125}$I- MoAb-Tg* radioactive tracers on Sephadex G-75 column were created (Figs. 2 and 3) respectively. The (Fig. 2) showed two peaks, that representing free radioactive $^{125}$I (8.9 %) and $^{125}$I-Tg* radioactive tracer (84.1 %) with a specific activity of 34 µCi/ µg. The (Fig. 3) illustrated two peaks that correspond to free radioactive $^{125}$I (10.8 %) and $^{125}$I-MoAb-Tg* radioactive tracer (76.5 %) with a specific activity 29.5 µCi/ µg. These results agree with those of Fraker and Speck [20].

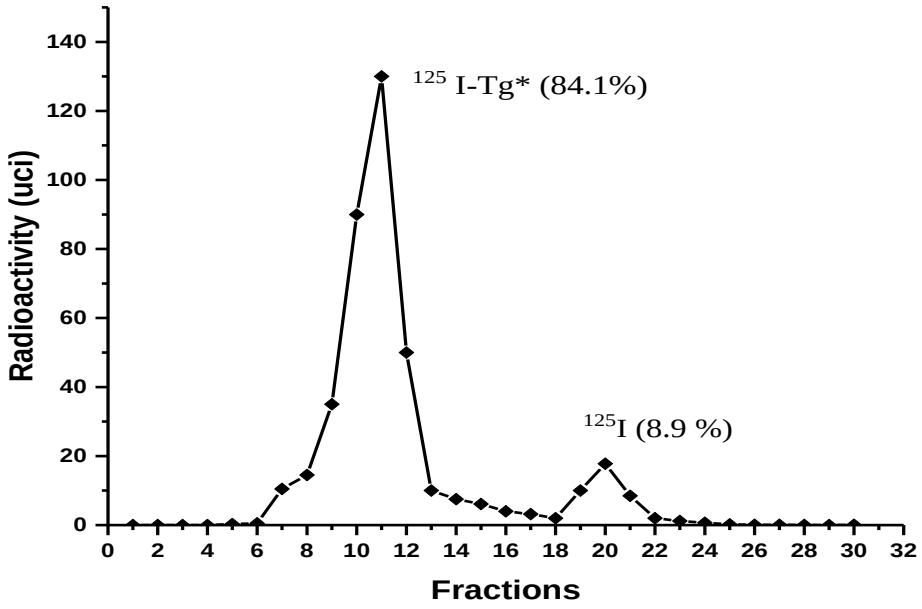


**Fig. (2): Purification profile of $^{125}$I-Tg* radioactive tracer using sephadex-75 column.**

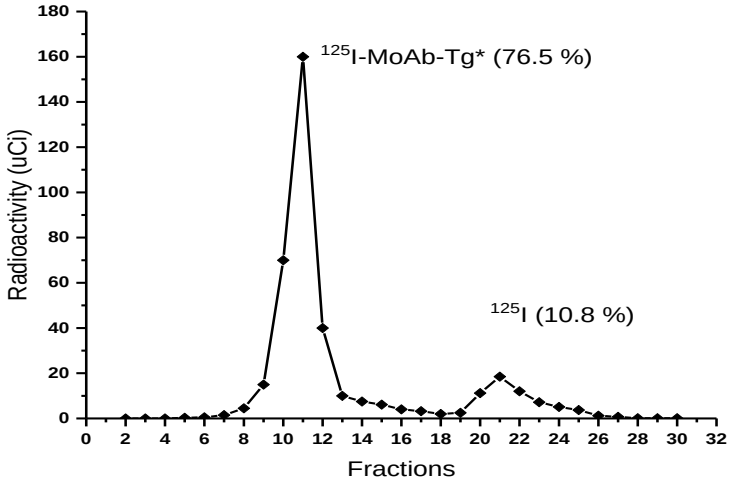


**Fig. (3): Purification profile of $^{125}$I- MoAb-Tg* radioactive tracer using sephadex-75 column.**

***Optimization of Tg-IRMA***

Following the fundamental stages of assay development is necessary to produce an assay that is sensitive and accurate. The design of the IRMA technique for Tg involved an investigation of the sample volume, incubation time, and washing procedure.

***Sample volume:*** Based on the findings, demonstrating the differences in binding between the low, medium, and high concentrations of Tg standards (1, 20, and 250 ng/ml) was most effectively achieved by using varied volumes (25, 50, and 100 µl). According to the findings presented in Table (2), the most significant binding differences were obtained with a sample volume of 100 µl.

**Table (2): Effect of sample volume on coated plate Tg-IRMA.**

| Tg standard (ng/ml) | Sample volume | | |
|---|---|---|---|
| | 25 µl | 50 µl | 100 µl |
| **1** | 0.23 | **0.4** | 0.41 |
| **20** | 3.5 | **6.3** | 6.1 |
| **250** | 24.2 | **40.8** | 36.5 |

The data are presented as % bound (B/TA).

***Incubation time:*** For a total of 24 hours that extend from 1, 2, 3 and 24 hours, the impact of the incubation time on this system was investigated at 37 ºC. As shown in Table (3), it was found that a suitable incubation time of 3 hours gave an optimal binding %.

**Table (3): Effect of incubation time on the coated plate Tg-IRMA.**

| Tg standard (ng/ml) | Incubation time | | | |
|---|---|---|---|---|
| | 1 hr. | 2 hr. | 3 hr. | 24 hr. |
| **1** | 0.29 | 0.32 | **0.43** | 0.45 |
| **20** | 4.7 | 5.7 | **6.5** | 6.6 |
| **250** | 27.8 | 33.1 | **41.5** | 41.5 |

The data are presented as % bound (B/TA).

***Washing procedure*:** The aim of the washing step was to solve the problem of the tracer's bound and free fractions possessing limited separation. After washing twice by 200 µl of washing buffer, as indicated in Table (4), the binding decreased and was stable as the washing step continued on. This result is consistent with earlier research [22].

**Table (4): Effect of washing volume on coated plate Tg-IRMA.**

| Tg standard (ng/ml) | Washing volume | | |
|---|---|---|---|
| | 200 µl once | 200 µl twice | 200 µl third |
| **1** | 0.65 | **0.39** | 0.39 |
| **20** | 7.9 | **6.2** | 6.1 |
| **250** | 53.5 | **40.7** | 40.6 |

The data are presented as % bound (B/TA).

Based on the prior results, the following can be used to summarize the optimal conditions for the Tg-IRMA system: To a locally coated plate (with anti-Tg), 100 µl of Tg standards or samples and 100 µl of $^{125}$I MoAb-Tg radioactive tracer (~ 140,000 cpm) were added. After mixing the plates, it were incubated for two hours at room temperature. The plates were washed twice by 200 µl of washing buffer after being decanted. The wells of the assay plate were cutting and counted by gamma counter. After the findings were calculated, the optimum standard curve for the solid phase coated plate Tg-IRMA system was shown, as (Fig. 4).

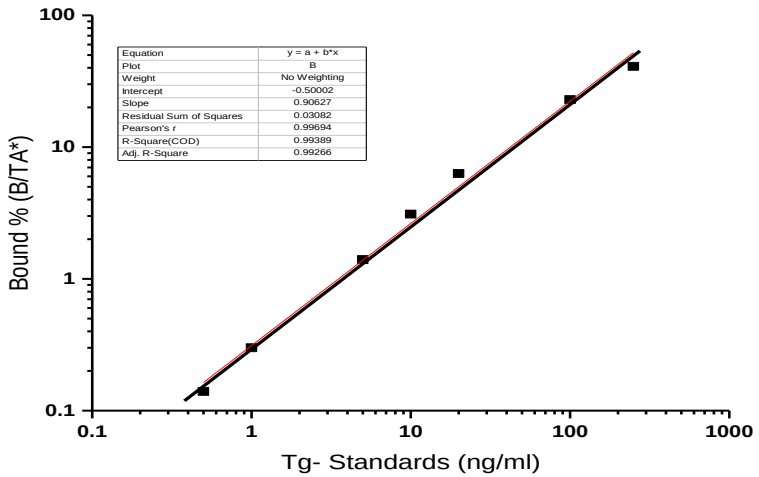


**Fig (4): Optimum standard curve for coated plate solid phase Tg-IRMA. (*TA: Total activity)**

### *Validity of Tg- IRMA*

A number of studies concerning qualities of performance has been examined in order to ensure the suggested test is valid and reliable.

***Sensitivity:*** By interpolating the mean minus two standard deviations (main - 2 SD) of twenty duplicates of the zero ng/ml Tg standards, the sensitivity or detection limit for the Tg-IRMA test was determined [23]. The sensitivity value for this investigation was determined at 0.1 ng/ml.

***Precision:*** By running three pooled samples with low, medium, and high Tg levels ten times (n=10) in the same test, the intra-assay precision was assessed. To further assess the accuracy of the inter-assay results, three more samples (n=10) were analyzed using a number of assays; the all results are listed in Table (5). The obtained results correspond with the findings of several studies [22, 23], which stated that the measured ligand concentration's CV (coefficient of variation) for the inter-assay should be less than 15%, and the same should be less than 10% for the intra-assay.

**Table (5): Precision profile (intra-assay and inter assay) for coated plate Tg-IRMA.**

| Samples | Intra-assay | | | Inter-assay | | |
|---|---|---|---|---|---|---|
| | Mean (ng/ml) | SD | CV% | Mean (ng/ml) | SD | CV% |
| **1** | 4.9 | 0.35 | 2.0 | 1.3 | 0.29 | 6.3 |
| **2** | 11.4 | 1.9 | 1.8 | 11.5 | 2.9 | 2.2 |
| **3** | 118.7 | 7.4 | 1.9 | 117.8 | 8.3 | 2.7 |

***Accuracy:*** To evaluate the assay's accuracy, recovery and dilution investigations were included in the study.

***Recovery - addition test:*** Recovery assays measure the concentrations in three samples of human serum from completely different diagnostic levels after the addition of a known amount of Tg. The recovery findings from the investigation are shown in Table (6) and are consistent with the data provided by Pillai and Bhandarkar [23], who said the recovery of the assay should be 100 ±15%. Recovery data presented in Table (6) indicates that the recovery % for Tg varied between 97.5% and 103.6%.

***Dilution - Linearity test:*** The data in Table (7) show the quantities for three different human samples in the assay medium at different dilutions to assess the assay's linearity. According to Edwards [24], non-linearity could be a sign of either weak matrix or inaccurate calibration. The findings of the present investigation demonstrate that, even when diluted, the analysis technique used in this research preserved a high degree of linearity.

**Table (6): Recovery test for coated plate Tg-IRMA.**

| Sample | Endogenous Tg (ng/ml) | Added Tg (ng/ml) | Expected value (E) | Observed value (O) | Recovery % (O/E) |
|---|---|---|---|---|---|
| **1** | 10 | 4.0 | 9.5 | 9.6 | 101.1 |
| | | 100 | 55 | 54.5 | 99.1 |
| | | 250 | 130 | 132.1 | 101.6 |
| **2** | 70 | 4.0 | 37 | 36.7 | 98.2 |
| | | 100 | 85 | 84.5 | 99.4 |
| | | 250 | 160 | 157.4 | 98.4 |
| **3** | 150 | 4.0 | 77 | 79.8 | 103.6 |
| | | 100 | 125 | 121.9 | 97.5 |
| | | 250 | 200 | 196.2 | 98.1 |

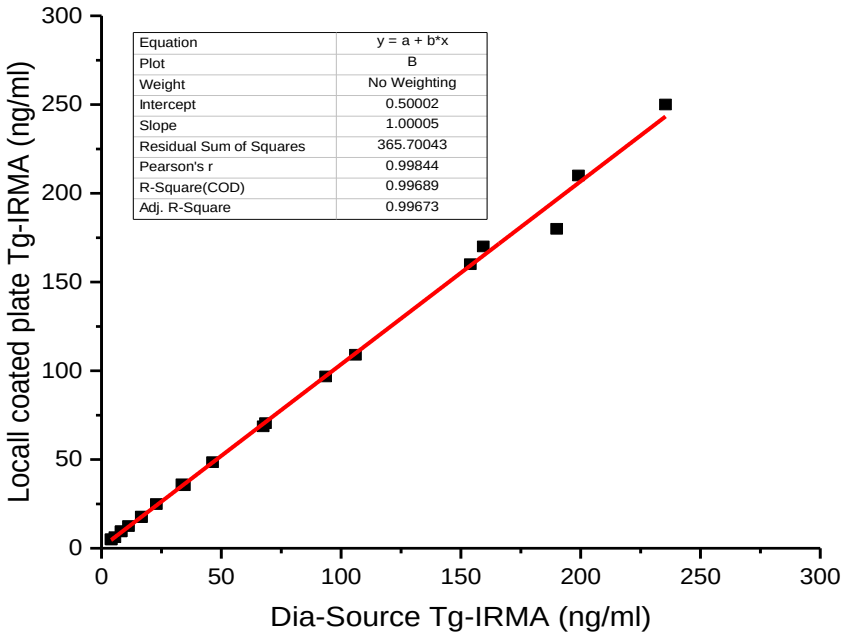


**Fig. (5): Regression line equation and correlation coefficient (r) between Tg values obtained by Tg-IRMA (Dia-Source) and present local method (solid phase Tg-IRMA system).**

**Table (7) Dilution test for coated plate Tg-IRMA.**

| Sample | Endogenous Tg (ng/ml) | Dilution factor | Expected value (E) | Observed value (O) | Recovery% (O/E) |
|---|---|---|---|---|---|
| **1** | 20 | 1:2 | 10 | 9.9 | 99 |
| | | 1:4 | 5 | 5.1 | 102 |
| | | 1:8 | 2.5 | 2.6 | 104 |
| **2** | 100 | 1:2 | 50 | 49.2 | 98.4 |
| | | 1:4 | 25 | 24.9 | 99.6 |
| | | 1:8 | 12.5 | 12.8 | 102.4 |
| **3** | 250 | 1:2 | 125 | 122.5 | 98 |
| | | 1:4 | 62.5 | 61.2 | 97.9 |
| | | 1:8 | 31.3 | 32.7 | 104.5 |

***Method comparison:***

In this study, the correlation coefficient was employed to assess the degree of relationship between two variables. A statistical comparison was made between the Tg results of 20 different human serum samples that were collected using commercially available kits (Dia Source, IRMA) and those obtained using a locally developed method (coated plate Tg-IRMA). The statistical analysis reveals that the results from the commercial kit (Dia-Source, IRMA) and the present system have an important correlation (r = 0.998), as illustrated in (Fig. 5).

## CONCLUSION

In conclusion, the coated plate thyroglobulin immunoradiometric technique (Tg-IRMA) is a simple and practical method that can be utilized in routine laboratory settings for measuring serum thyroglobulin levels, diagnosing hyperthyroidism, monitoring thyroid cancer treatment, and detecting recurrences. This study highlights the importance of the immunoradiometric system for thyroglobulin, which is widely recognized as a crucial radiological diagnostic tool in the medical field.

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