المجلة العراقية
لبحوث السوق وحماية المستهلك

المجلد (5) العدد (1)
لسنة 2013

# Effect of Room Temperature Variations on ELISA Results

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## Abstract

**This study was designed to investigate the effect of room temperature variations on ELISA results. Sixty serum samples were collected from healthy and patients persons, distributed as three groups; 1st. group included 27 samples for thyroids hormones, 2nd.group included 23 samples for some fertility hormones and the 3rd. group included 10 samples for ferritin. The concentration of each hormone was estimated at 25 °C and compared twice; once with the standards of the corresponding hormone were measured at 21 °C; and the other with the standards of the corresponding hormone were measured at 25 °C. The results showed that there were variations in the concentrations of each hormone at the two different temperatures. Regarding thyroids and fertility hormones, the optical density of the standards concentrations at 25 °C were higher than these at 21 °C, while in ferritin test, the optical density of the standards concentrations at 25 °C were lower than these at 21 °C. It could be concluded that the variation in room temperature play a role in estimation of the exact concentrations of hormones in stability of other influencing factors.**

**Key words**: **ELISA, Temperature variations, thyroids hormones, fertility hormones**

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# تأثير تغيرات درجة حرارة الغرفة على نتائج تقنية الامتزاز المناعي بالإنزيم المرتبط

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## الخلاصة

صممت هذه الدراسة للتقصي عن تأثير تغيرات درجة حرارة الغرفة على نتائج تقنية الامتزاز المناعي بالانزيم المرتبط. جمعت ستين عينة مصل من اشخاص أصحاء ومرضى، وزعت العينات على ثلاث مجاميع ضمت المجموعة الاولى 27 عينة لدراسة هورمونات الغدة الدرقية، والمجموعة الثانية 23 عينة لدراسة بعض هورمونات الخصوية، وتضمنت المجموعة الثالثة 10 عينات لدراسة انزيم الفيريتين. تم قياس تراكيز كل من الهورمونات بدرجة حرارة 25 °م وتمت مقارنتهامع تراكيز المعايير الخاصة لكل هورمون مرتين: المرة الاولى مع تراكيز المعايير مقاسة بدرجة 21°م والاخرى مع تراكيز المعايير مقاسة بدرجة 25 °م . بينت النتائج وجود تغيير في تراكيز الهورمونات باختلاف درجة الحرارة. بالنسبة لهورمونات الغدة الدرقية وهورمونات الخصوبة عند درجة الحرارة 25 °م كانت الامتصاصية لتراكيز المعايير أعلى من قرائتها عند درجة 21 °م. أما بالنسبة للفيريتين فأن الامتصاصية لتراكيز المعايير عند درجة الحرارة 25 °م أقل من قرائتها عند درجة الحرارة 21 °م. يمكن أن نستنتج بان تغيرات درجة حرارة الغرفة تلعب دورا مهما في تقدير التركيز المضبوط للهورمونات عند استقرار بقية العوامل المؤثرة على اجراء الفحص.

الكلمات المفتاحية: تقنية الامتزاز المناعي، تغيرات درجة الغرفة، هورمونات الغدة الدرقية، هورمونات الخصوبة.

## Introduction

ELISA which stands for (Enzymes Linked Immunosorbent Assay) represent one of the most valuable techniques can be performed to evaluate either the presence of antigen or the presence of antibody in a sample, it is a useful tool for determining serum antibody concentrations (such as with the HIV test or West Nile virus). It has also found applicapable in the food industry in detecting potential food allergens, such as milk, peanuts, walnuts, almonds and eggs. ELISA can also be used in toxicology as a rapid presumptive screen for certain classes of drugs (7). The reaction between antigens and antibodies relies on their close proximity; during ELISA, this is affected by their respective concentrations, distribution, time and temperature of incubation, and pH (buffering conditions). In any interaction, the avidity of the antibodies for the particular antigens is also important (4). Two types of incubation conditions are common; incubation of rotating plates (with shaking) and incubation of stationary plates. Temperatures are among these stationary conditions affect and required for successful ELISAs and are therefore should be discussed separately (8).

Room temperature (also referred to as ambient temperature) is a common term to denote a certain temperature within enclosed space to which human beings are accustomed. Room temperature is thus often indicated by general human comfort, with the common range of 18°C (64 °F) to 23°C (73 °F), though differences in climate may acclimate people to higher or lower temperatures for instance, 25.5 °C (78 °F) could be a common temperature for some people. The term may also refer to a certain temperature within settings of scientific experiments and calculations (1). For human comfort, desirable room temperature greatly depends on individual needs and various other factors. According to the West Middle and Public Health Observatory (UK), 21 °C (70 °F) is the recommended living room temperature, whereas 18 °C (64 °F) for bedroom temperature (2). For scientific calculations, room temperature is taken to be roughly 20 to 23.5 °C, with an average of 21 °C. For numerical convenience, either 20 °C is often used. However, room temperature is not a precisely defined scientific term as opposed to Standard Temperature and Pressure, which has several, slightly different definitions (3).

All companies manufacturing ELISA kits give a range for room temperature from (18-25) °C without determining the exact recommended temperature especially when the required tests have no need to be incubated at 37 °C. On the other hand, all working laboratories prepare their standards for specific test for once only (just at the first use of the kit). On the strength mentioned above, this research was designed to detect the effect of room temperature variations on ELISA results in stability of other factors (for instance, incubation time, incubation at dark or light, and rotation or shaking).

## Materials and Methods

**Samples Collection:**

Sixty samples were collected from private laboratory in Baghdad governorate during a period lasting for four months distributed as 27 samples for thyroids hormones, 23 sample for Fertility hormones and 10 samples for ferritin.

**Methods:**

All assays in this research were performed in duplicates according to (9) dependent by Monobind Company (USA).

**Thyroids Hormones Assay:**

Regarding T3, T4 and TSH hormones, a quantity of 50 µl, 25 µl and 50 µl respectively from specimen and standards were added to Ab-coated micro wells specific for each hormone on two separate plates; each plate contains the same order of wells of the three hormones followed by addition of 100 µl of specific Enzyme conjugate. One plate was incubated in 21 °C and the other was incubated at 25 °C for 60 min. after elapsing the incubation period, the contents of each plate were discarded and washed with a buffer, a quantity of 100 µl substrate was added to each well and reincubated at the same conditions for 15 min. a portion of 50 µl stop solution was added to each well, the results were read at 492 nm by micro plate reader. All samples and standards were performed in duplicates.

**Fertility Hormones Assay:**

In testosterone and prolactin hormones, a quantity of 10 µl and 25 µl respectively from specimen and standards were added to Ab-coated micro wells specific for each hormone on two separate plates;

each plate contains the same order of wells. Regarding testosterone, 50 µl enzyme conjugate was added followed by addition of 50 µl testosterone biotin. While in prolactin assay, 100 µl of specific Enzyme conjugate was added directly on specimen. One plate was incubated in 21 °C and the other was incubated at 25 °C for 60 min. after elapsing the incubation period, the contents of each plate were discarded and washed with a buffer, a quantity of 100 µl substrate was added to each well and reincubated at the same conditions for 20 min. a portion of 50 µl stop solution was added to each well, the results were read at 492 nm by micro plate reader. All samples and standards were performed in duplicates.

**Ferritin Assay:**

Two plate of ferritin Ab coated wells were treated with 25 µl of specimens and ferritin standards, followed by addition of 50 µl ferritin biotin, one plate was incubated at 21°C and the other was incubated at 25 °C for 30 min, then 50 µl of ferritin enzyme conjugate was added to each well and re-incubated at the same condition for 30 min. after elapsing the incubation period, the contents of each plate were discarded and washed with a buffer, a quantity of 100 µl substrate was added to each well and reincubated at the same conditions for 15 min. a portion of 50 µl stop solution was added to each well, the results were read at 492 nm by micro plate reader. All samples and standards were performed in duplicates.

**Calculations:**

The results of each hormone for all tested persons were measured in duplicate at 25 °C and compared with standards measured in duplicate at 21 °C and 25 °C.

**Results:**

The data showed that variation in room temperature points significantly affect ELISA results.

**Thyroids Hormones:**

The standards of thyroids hormones (T3, T4 and TSH) showed different readings at different room temperatures, as shown in (figures 1, 2, 3, 4, 5 and 6).

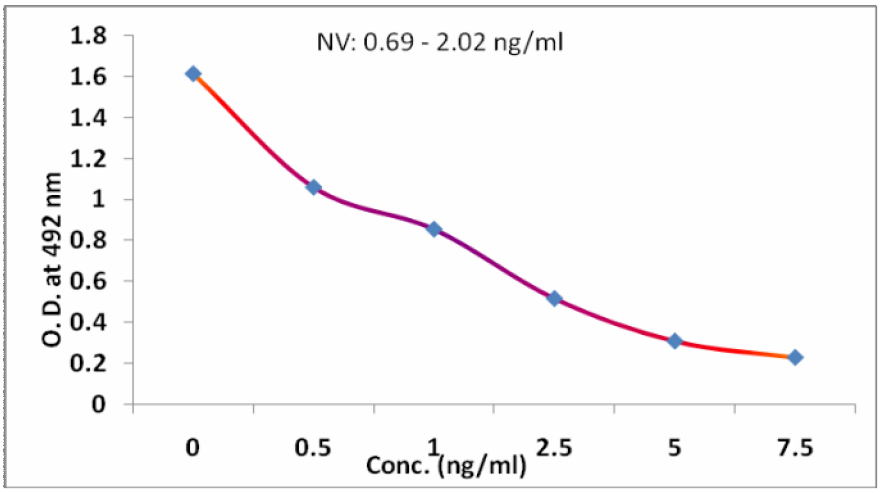


**Figure (1):** Standard Curve of T3 Hormone at 21 °C.

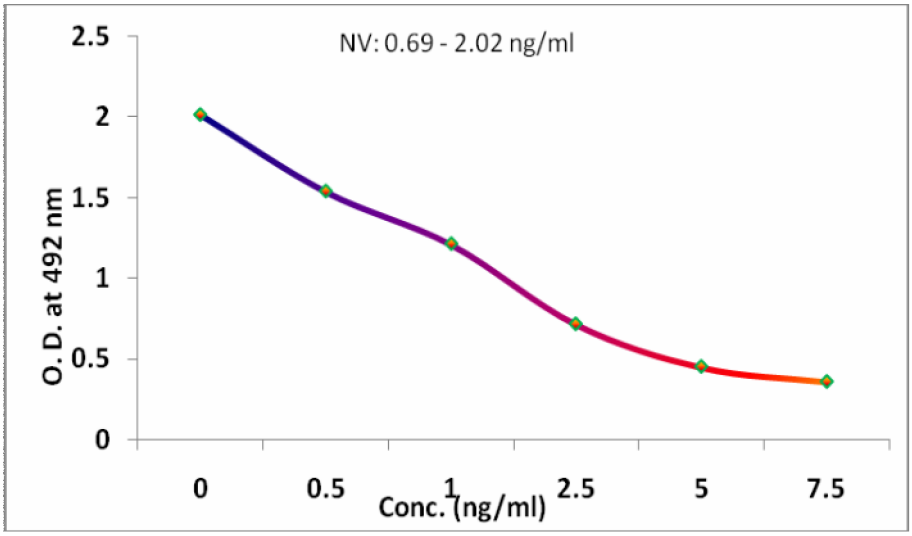


**Figure (2):** Standard Curve of T3 Hormone at 25 °C**.**

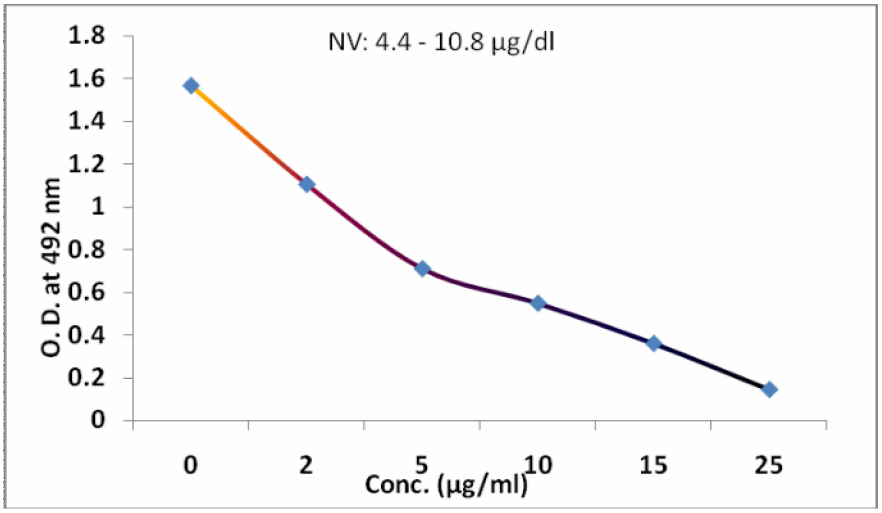


**Figure (3):** Standard Curve of T4 Hormone at 21 °C.

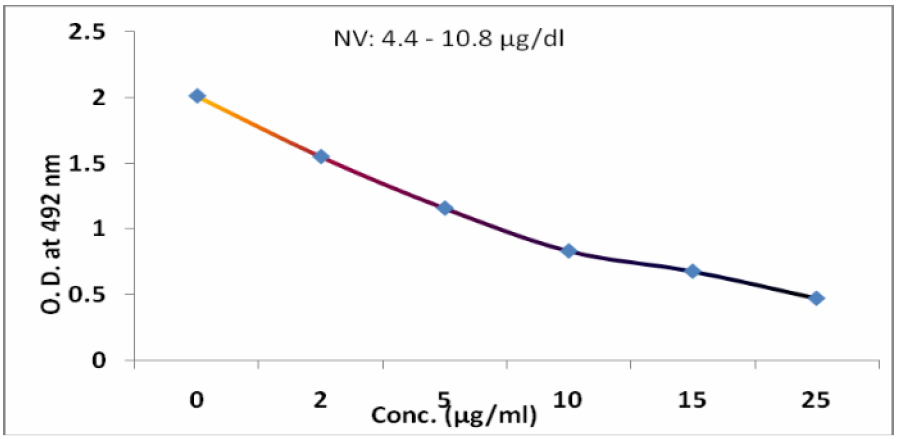


**Figure (4):** Standard Curve of T4 Hormone at 25 °C.

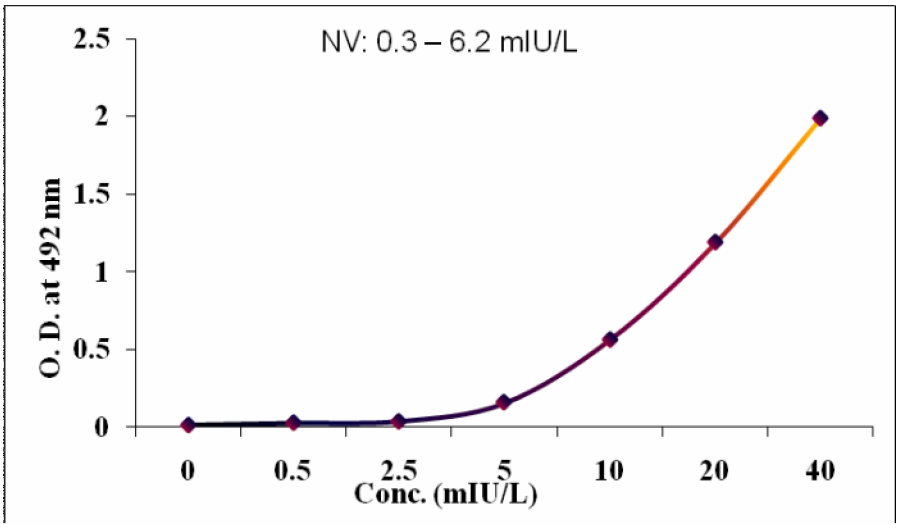


**Figure (5):** Standard Curve of TSH Hormone at 21 °C.

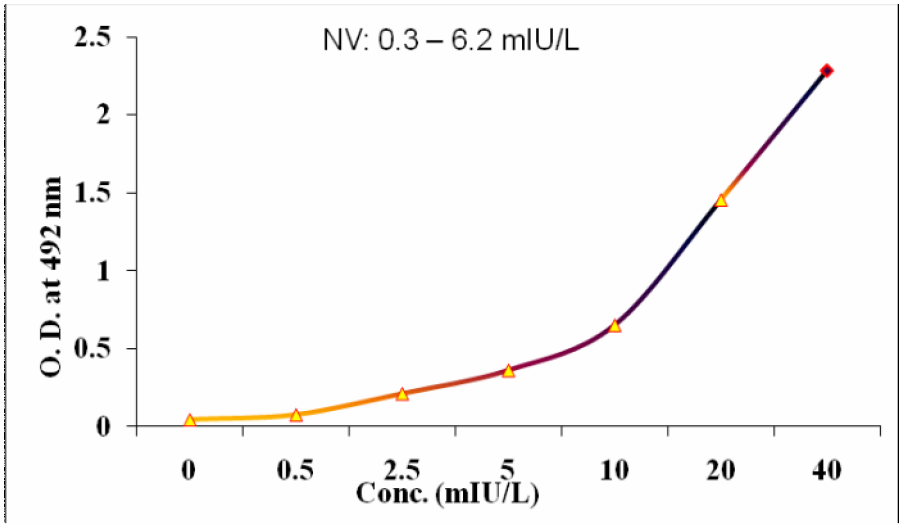


**Figure (6):** Standard Curve of TSH Hormone at 25 °C.

When room temperature increase, the optical density (OD) increase for the same standard concentration for T3, T4 and TSH.

Among 27 samples for thyroid hormones, we select only 10 samples for comparison; 5 samples as normal cases their OD shown in (table 1). These samples were referred to as healthy persons (HP). While the other 5 samples were abnormal cases referred to as patient persons (PP).

**Table (1):** T3, T4 and TSH OD Values of Healthy Persons (HP) at 25°C.

| Persons No. | OD Values of | | |
|---|---|---|---|
| | **T3** | **T4** | **TSH** |
| HP 1 | 1.347 | 0.899 | 0.289 |
| HP 2 | 1.415 | 0.937 | 0.331 |
| HP 3 | 1.231 | 0.827 | 0.148 |
| HP 4 | 1.318 | 0.768 | 0.153 |
| HP 5 | 1.308 | 0.870 | 0.064 |

When comparing the OD of each person for the three hormones with OD of the standards of the same hormone at 25 °C, the results will be normal; since the concentrations of T3, T4 and TSH for HP1 0.8 ng/ml, 5.8 µg/dl and 4.1 mIU/L respectively, which are within normal range. Also the same will be for other healthy persons; HP 2: T3, T4 and TSH concentrations are 0.73 ng/ml, 5.3 µg/dl and 4.9 mIU/L respectively, HP 3: T3, T4 and TSH concentrations are 1.0 ng/ml, 6.7 µg/dl and 1.8 mIU/L respectively. HP 4: T3, T4 and TSH concentrations are 0.82 ng/ml, 7.8µg/dl and 1.87 mIU/L respectively. HP 5: T3, T4 and TSH concentrations are 0.87 ng/ml, 6.3 µg/dl and 0.35 mIU/L respectively. All these results are considered normal when compared with reference range of the corresponding hormone.

While if the same OD of the same patients were compared with OD of standards of the same hormones (T3, T4 and TSH) but these OD were measured at 21°C, the results will be abnormal since the concentrations of T3, T4 and TSH for HP1 0.22 ng/ml, 4.4 µg/dl and 6.8 mIU/L respectively, which are now abnormal results. Also the same will be for other healthy persons; HP 2: T3, T4 and TSH concentrations are 0.19 ng/ml, 3.8 µg/dl and 7.3 mIU/L respectively, HP 3: T3, T4 and TSH concentrations are 0.3 ng/ml, 4.7 µg/dl and 4.8 mIU/L respectively. HP 4: T3, T4 and TSH concentrations are 0.21 ng/ml, 4.9

µg/dl and 5.2 mIU/L respectively. HP 5: T3, T4 and TSH concentrations are 0.2 ng/ml, 4.5 µg/dl and 2.9 mIU/L respectively. In cases of P2, P3 all hormones concentrations are abnormal while in case P4 and P5, T4 looks to be at the lower border line and give suspect to thyroid disorder. Also in case P5, TSH looks to be within normal range while it is at the lower limit of the normal range.

On the other hand, the OD of the patients persons (PP) their OD measured at 25 °C explained in (table 2) when compared with OD of the same hormones standards (T3, T4 and TSH) also measured at 25 °C, their hormones concentration were; for PP1: 3.9 ng/ml, 20.0 µg/dl and 0.1 mIU/L respectively, which are abnormal results. Also the same will be for other patients persons; PP 2: T3, T4 and TSH concentrations are 2.5 ng/ml, 13.5 µg/dl and 0.25 mIU/L respectively, PP 3: T3, T4 and TSH concentrations are 0.45 ng/ml, 4.9 µg/dl and6.8 mIU/L respectively. PP 4: T3, T4 and TSH concentrations are 3.7 ng/ml, 13.8 µg/dl and 0.6 mIU/L respectively. PP 5: T3, T4 and TSH concentrations are 4.1 ng/ml, 25.0 µg/dl and 0.3 mIU/L respectively. All these results are abnormal when compared with the normal values of each hormone except T4 concentration of PP3 and TSH for PP 4.

**Table (2):** T3, T4 and TSH OD Values of Patients Persons (PP) at 25°C.

| Persons No. | OD Values of | | |
|---|---|---|---|
| | T3 | T4 | TSH |
| PP 1 | 0.571 | 0.373 | 0.029 |
| PP 2 | 0.716 | 0.526 | 0.043 |
| PP 3 | 1.614 | 0.974 | 0.481 |
| PP 4 | 0.580 | 0.409 | 0.059 |
| PP 5 | 0.542 | 0.270 | 0.044 |

When comparing these OD with the same standards their OD measured at 21°C, many results will seems to be normal or increased over the exact measured value and give false positive or increased concentrations, since their results will be for PP1: 2.1 ng/ml, 14.1 µg/dl and 0.6 mIU/L respectively, which show that both T3 and T4 concentrations are abnormal but seems to be at the border lines, while TSH concentrations looks to be normal. Also the same will be for other patients persons; PP 2: T3, T4 and TSH concentrations are 1.6 ng/ml, 8.5 µg/dl and 2.5 mIU/L respectively, these results are normal when

compared with the reference range of the tested hormones. PP 3: T3, T4 and TSH concentrations are 0.05ng/ml, 3.9 µg/dl and 9.3 mIU/L respectively which give false concentrations that affect medications courses. PP 4: T3, T4 and TSH concentrations are 2.4 ng/ml, 12.0 µg/dl and 2.9 mIU/L respectively these results seem to be at the lower border line for both T3 and T4 while TSH concentration looks to be normal. PP 5: T3, T4 and TSH concentrations are 2.2 ng/ml, 17.5 µg/dl and 2.8 mIU/L respectively, in which T3 looks at the lower border line and TSH is within normal range.

**Fertility Hormones:**

Fertility hormones studied in this research were testosterone and prolactin since they represent the most common required tests by physician. The standards of testosterone and prolactin hormones showed different readings at different room temperatures, as shown in (figures 7, 8, 9 and 10), when room temperature increase, the optical density (OD) increase for the same standard concentration.

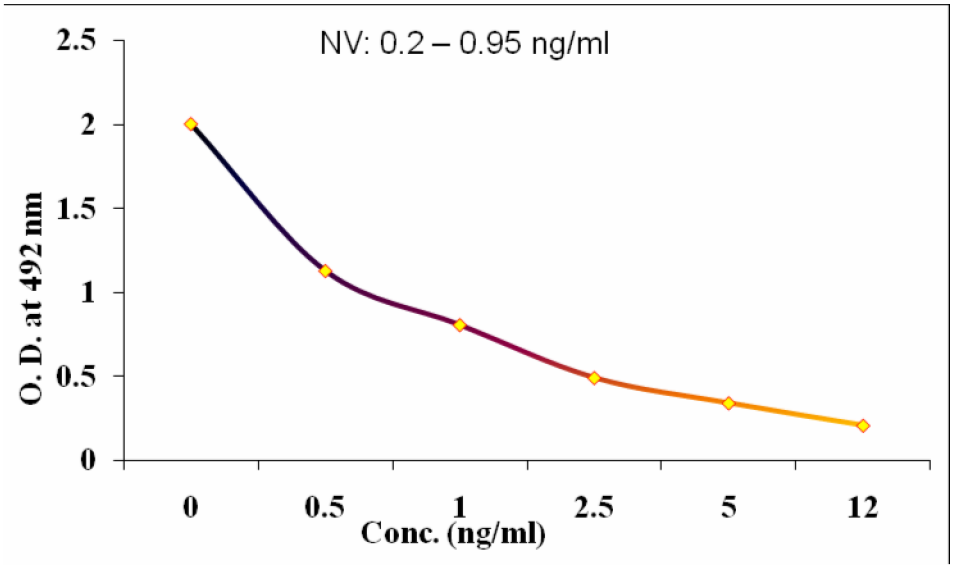


**Figure (7):** Standard Curve of Testosterone Hormone at 21 °C.

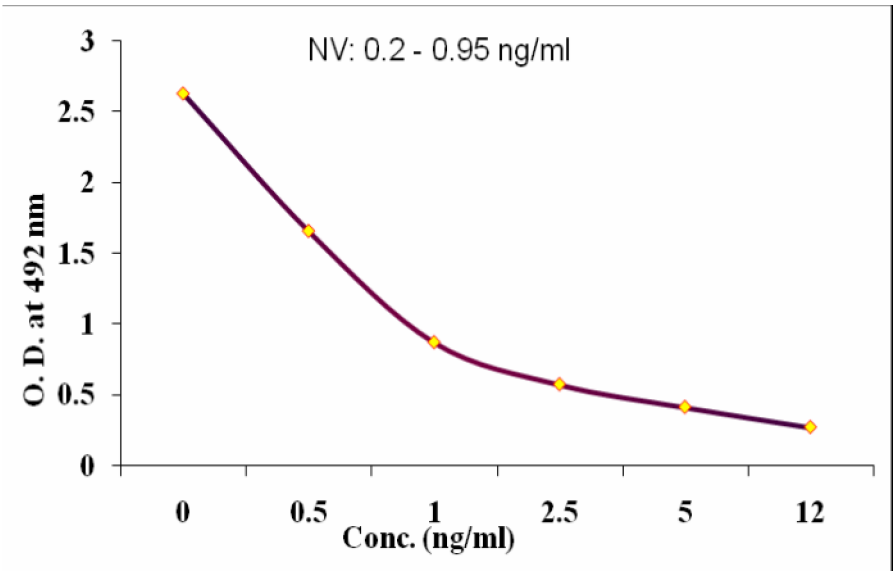


**Figure (8):** Standard Curve of Testosterone Hormone at 25 °C.

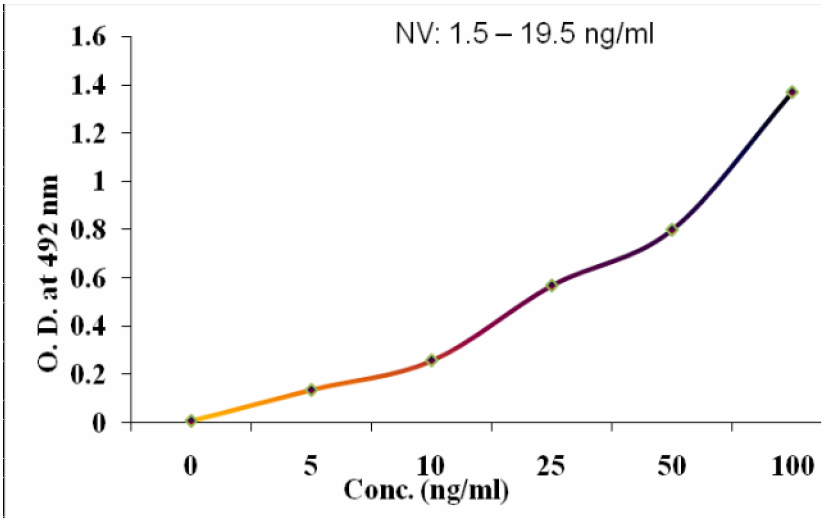


**Figure (9):** Standard Curve of Prolactine Hormone at 21 °C.

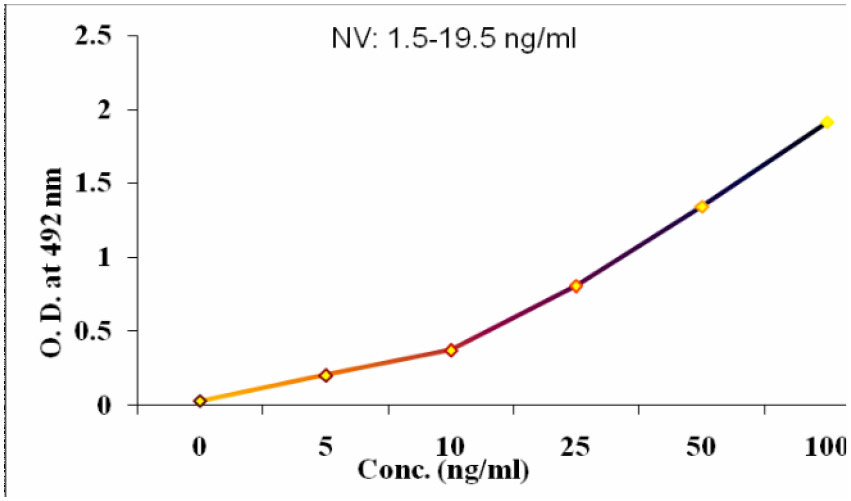


**Figure (10):** Standard Curve of Prolactin Hormone at 25 °C.

Among 23 samples for testosterone and prolactin hormones, we select only 10 female samples for comparison; 5 samples as normal cases their OD shown in (table 3), these samples were referred to as healthy female (HF), while the other 5 samples were abnormal cases referred to as patient female (PF).

**Table (3):** OD Values Testosterone and Prolactin of Healthy Females (HF) at 25 °C.

| Persons No. | OD Values of | |
|---|---|---|
| | Testosterone | Prolactin |
| HF 1 | 1.336 | 0.489 |
| HF 2 | 1.836 | 0.391 |
| HF 3 | 1.857 | 0.461 |
| HF 4 | 1.637 | 0.479 |
| HF 5 | 0.916 | 0.472 |

When comparing the OD of each person for the two hormones with OD of the standards of the same hormone at 25 °C, the results will be normal; since the concentrations of testosterone and prolactin for HF1 0.57 ng/ml and 19.37 ng/ml respectively, which are within normal

range. Also the same will be for other healthy females; HF 2: testosterone and prolactin concentrations are 0.4 ng/ml and 14.1 ng/ml respectively, HF 3: testosterone and prolactin concentrations are 0.35 ng/ml and 17.0 ng/m respectively. HF 4: testosterone and prolactin concentrations are 0.46 ng/ml and 18.6 ng/ml respectively. HF 5: testosterone and prolactin concentrations are 0.93 ng/ml and18.3 ng/ml respectively. All these results are considered normal when compared with reference range of the corresponding hormone.

While if the same OD of the same females were compared with OD of standards of the same hormones (testosterone and prolactin) but these standards OD were measured at 21°C, the results will be abnormal since the concentrations of testosterone and prolactin for HF1 0.3 ng/ml and 21.0 ng/ml respectively, which are now abnormal results. Also the same will be for other healthy females; HF 2: testosterone and prolactin concentrations are 0.12 ng/ml and 17.0 ng/ml respectively, HF 3: testosterone and prolactin concentrations are 0.11 ng/ml and 20.0 ng/ml respectively. HF4 testosterone and prolactin concentrations are 0.2 ng/ml and 20.8 ng/ml respectively. HF 5: testosterone and prolactin concentrations are 0.8 ng/ml and 20.5 ng/ml respectively. In all cases mentioned above most hormones concentrations are abnormal except in case F1 in which testosterone seems to be at the lower border line whereas it is normal, and F2, prolactin concentration looks to be at normal but this may give confusion when the patient is under treatment.

On the other hand, the OD of the patients females (PF) their OD measured at 25 °C explained in (table 4) when compared with OD of the same hormones standards (testosterone and prolactin) also measured at 25 °C, their hormones concentration were; for PF1: (1.5ng/ml and 35.5 ng/ml) respectively, which are abnormal results. Also the same will be for other patients females; PF 2: testosterone and prolactin concentrations are 1.2 ng/ml and 23.0 ng/ml respectively, PF 3: testosterone and prolactin concentrations are 1.1 ng/ml and 28.2 ng/ml respectively. PF 4: testosterone and prolactin concentrations are 0.1 ng/ml and 22.0 ng/ml respectively. PF 5: testosterone and prolactin concentrations are 0.25 ng/ml and 19.7 ng/ml respectively. All these results are abnormal when compared with the normal values of each hormone except testosterone concentration of PF5 which were at the lower border line.

**Table (4):** OD Values Testosterone and Prolactin of Patients Females (PF) at 25 °C.

| Persons No. | OD Values of | |
|---|---|---|
| | Testosterone | Prolactin |
| PF 1 | 0.684 | 0.975 |
| PF 2 | 0.697 | 0.746 |
| PF 3 | 0.813 | 0.896 |
| PF 4 | 2.14 | 0.723 |
| PF 5 | 1.946 | 0.656 |

When comparing these OD with the same standards their OD measured at 21°C, many results will seems to be normal or increased over the exact measured value and give false positive or increased decreased concentrations depending on the actual concentration in the patients serum, since their results will be for PF1: testosterone and prolactin concentrations are 1.1 ng/ml and 69.2 ng/ml respectively, which show that testosterone concentration is seems to be at the upper normal limit while prolactin concentration is highly increased. PF 2: testosterone concentrations are 0.95 ng/ml and 43.8 ng/ml respectively, these results show that testosterone concentration is at the upper normal limit while prolactin concentration is highly increased. PF 3: testosterone and prolactin concentrations are 0.9 ng/ml and 58.7 ng/ml respectively which give false concentrations for testosterone that is actually abnormal but here seems to be normal. PF 4: testosterone and prolactin concentrations are 0.02 ng/ml and 42.3 ng/ml respectively which show a highly reduced concentration of testosterone. PF 5: testosterone and prolactin concentrations are 0.8 ng/ml and 34.7 ng/ml respectively, in which testosterone looks normal while prolactin in all cases is highly increased and this will give confusion regarding medication courses.

**Ferritin:**

The standards of ferritin showed different readings at different room temperatures, as shown in (figures 11 and 12), when room temperature increase, the optical density (OD) decrease for the same standard concentration.

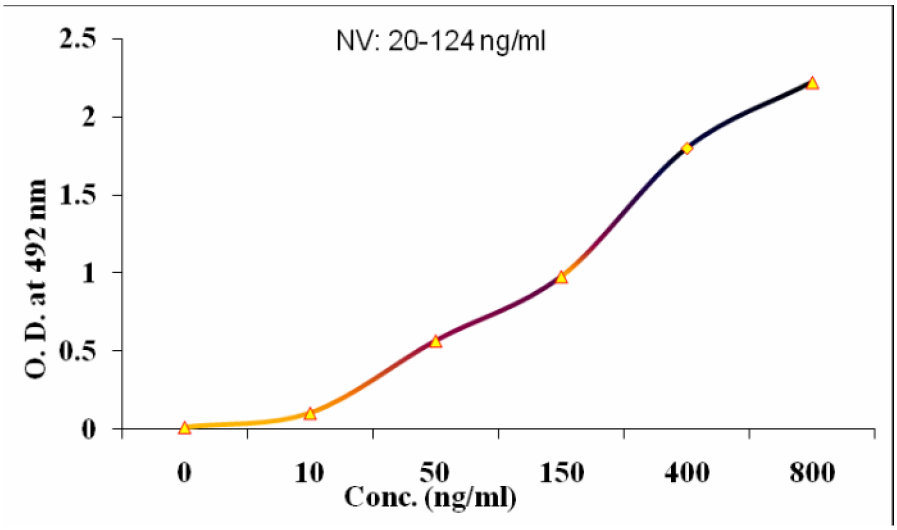


**Figure (11):** Standard Curve of Ferritin at 21 °C.

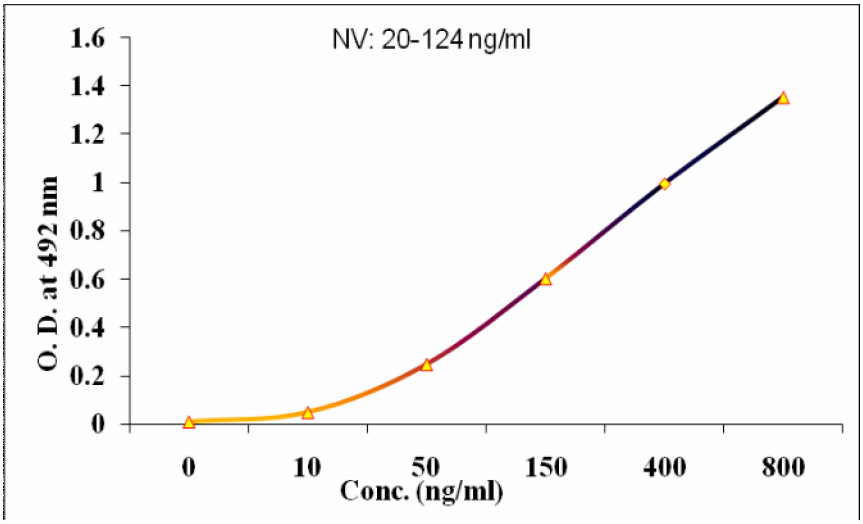


**Figure (12):** Standard Curve of Ferritin at 25 °C.

Among 10 females for ferritin test, we select 5 samples as normal cases their OD shown in (table 5), these samples were referred to as healthy female (HF). While the other 5 samples were abnormal cases referred to as patients females (PF).

**Table (5):** OD Values Ferritin Healthy Female (HF) and Patients Female (PF) at 25 °C.

| Persons No. | OD | | Persons No. | OD |
|---|---|---|---|---|
| HF 1 | 0.245 | | PF 1 | 0.114 |
| HF 2 | 0.216 | | PF 2 | 0.694 |
| HF 3 | 0.323 | | PF 3 | 1.341 |
| HF 4 | 0.228 | | PF 4 | 0.792 |
| HF 5 | 0.221 | | PF 5 | 1.332 |

When comparing the OD of each female for ferritin with OD of the standards at 25 °C, the results were normal; since the concentrations of ferritin for HF1was 49.5 ng/ml. Also the same will be for other healthy females; HF 2: a ferritin concentration was 41.0 ng/ml, HF 3: ferritin concentration was 68.8 ng/ml. HF 4: ferritin concentration was 44 ng/ml. HF 5: ferritin concentration was 39 ng/ml. All these results are considered normal when compared with reference range.

While if the same OD of the same females were compared with OD of standards measured at 21°C, the result for HF1 was 22.5 ng/ml. For HF 2: ferritin concentration was 19.0 ng/ml, for HF 3: ferritin concentration was 27.6 ng/ml. For HF4: ferritin concentration was 25.6 ng/ml. HF 5: ferritin concentration was 20.5 ng/ml In all cases mentioned above ferritin concentration looked to be at the lower limit of normal value which confuse medication course.

On the other hand, the OD of the patients females (PF) their OD measured at 25 °C explained in (table 5) when compared with OD of the standards also measured at 25 °C, their hormones concentration were; for PF1: 18.0 ng/ml. Also the same for other patients; PF 2: ferritin concentration was 148.5 ng/ml, PF 3: ferritin concentration was 780 ng/ml, for PF 4: ferritin concentration was 168.9 ng/ml. and for PF 5: ferritin concentration was 784 ng/ml. All these results are abnormal when compared with the normal range.

While if the same OD of the same females were compared with OD of standards measured at 21°C, the result for PF1 was 9.0 ng/ml which is severely reduced. For PF 2: ferritin concentration was 63.25 ng/ml which appeared as normal. For PF 3: ferritin concentration was 212.6 ng/ml and for PF 5: ferritin concentration was 307 ng/ml even they seem to be high, but they severely reduced below the actual concentration and confuses the medication care. For PF4: ferritin concentration was 56.4 ng/ml which looks to be normal.

## Discussion

Varying temperatures in the performance of the ELISA can cause results variation. It is advisable; therefore, that substrates are added at a defined temperature and the plates were incubated under uniform conditions normally room temperature. Each laboratory should be assessed since temperature can vary greatly indifferent periods. The best practice is to add substrate solutions at a defined temperature obtained by using solutions heated to (or cooled) to that defined temperature. This is particularly important when attempting to standardize assays among operators and laboratories in which a fixed time for stopping an assay is used (10).

Assays may be geared to stationary conditions, although the exact times and temperatures of incubation may vary. The temperatures for incubation are most commonly 37 °C, room temperature (on the bench), and 4 °C. Usually the time of incubation under stationary conditions reflects which incubation temperature is used. Therefore, at 4°C, a longer incubation might be given (over-night). In general, most incubation for stationary assays involving the reaction of antigen and antibodies are 1–3 h at 37 °C (11). Sometimes these conditions are combined so that one reagent is added for, say, 2 h at 37 °C followed by one overnight at 4 °C, usually because this produces a convenient work schedule (13). When incubation is performed at room temperature; care must be taken to monitor possible seasonal variations in the laboratory, since temperatures can be quite different, particularly in non-temperate countries (12). Direct sunlight should also be avoided, as must other sources of heat, such as from machinery in the laboratory. Also, how the plates are placed during stationary incubation should be considered; ideally, they should be separated and not stacked this can be a primary cause of operator-to-operator variation. Depending on the temperature, the higher the temperature, the greater the rate antibody-

antigen binding, so increasing the temperature may have a deleterious effect on antigen(s) (5).

The rate of the hydrophobic interactions depends on the temperature: the higher the temperature, the greater the rate. There are many variations on incubation conditions. It must be remembered that all factors affect the coating, and thus a higher concentration of protein may allow a shorter incubation time as compared with a lower concentration of the antigen for a longer time. The most usual regimes involve incubation at 37 °C for 1–3 h or over-night at 4 °C, or a combination of the two, or incubation (more vaguely) at room temperature for 1–3 h (6). There are many more variations, and ultimately, each scientist must titrate a particular antigen to obtain a standardized regime. Increasing the temperature may have a deleterious effect on antigens in the coating stage (10).

We see that each laboratory should establish a new standard curve for each run, but this will be costly and time consuming, the alternative is to check room temperature when standard curve was established and maintain this temperature to be applied at the next run, this could be done by air conditioner at specific temperature.

## Conclusion

Room temperature variations is among the factors affecting ELISA results, since the standards measured at 25 °C differ from these measured at 21 °C, in the present study, standard curves demonstrated that the results measured at 25 °C are may be more precise than those measured at 21 °C.

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