Mansoura National University
Faculty of Medicine

Level: 2

Semester: 4

Practical Medical biochemistry

Laboratory evaluation of thyroid hormones

Contents

- Case scenario.
- Introduction about thyroid gland and thyroid hormones.
- Hypothalamic-pituitary-thyroid axis.
- Disorders of thyroid gland.
- Laboratory evaluation of thyroid hormones.
- Interpretation of results of laboratory tests of thyroid hormones.

Learning Outcomes (LOs)

At the end of this section, the students should be able to:

- Demonstrate methods of laboratory evaluation of thyroid hormones
- Interpret the results of the laboratory tests of thyroid hormones

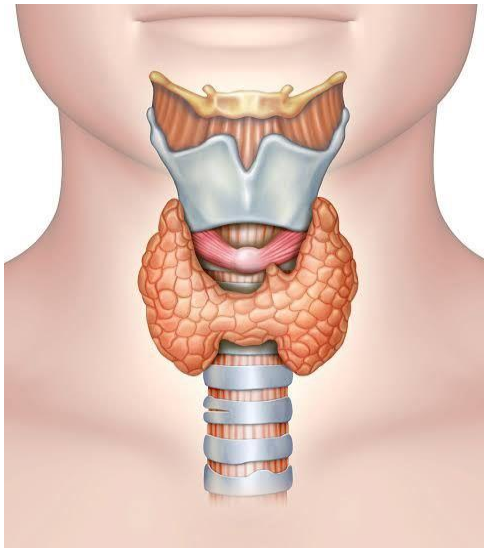
Case scenario

A 30-year-old woman presents with anxiety complaining of lack of sleep and fatigue. Symptoms were excessive sweating, diarrhea, heat intolerance and weight loss despite good appetite. Routine vital signs indicate mild tachycardia. A TSH test and a complete blood count were ordered.

1. **What is your probable diagnosis?**
2. **What do you expect as regard TSH blood level?**
3. **Suggest other biochemical investigations to confirm your diagnosis.**

Introduction

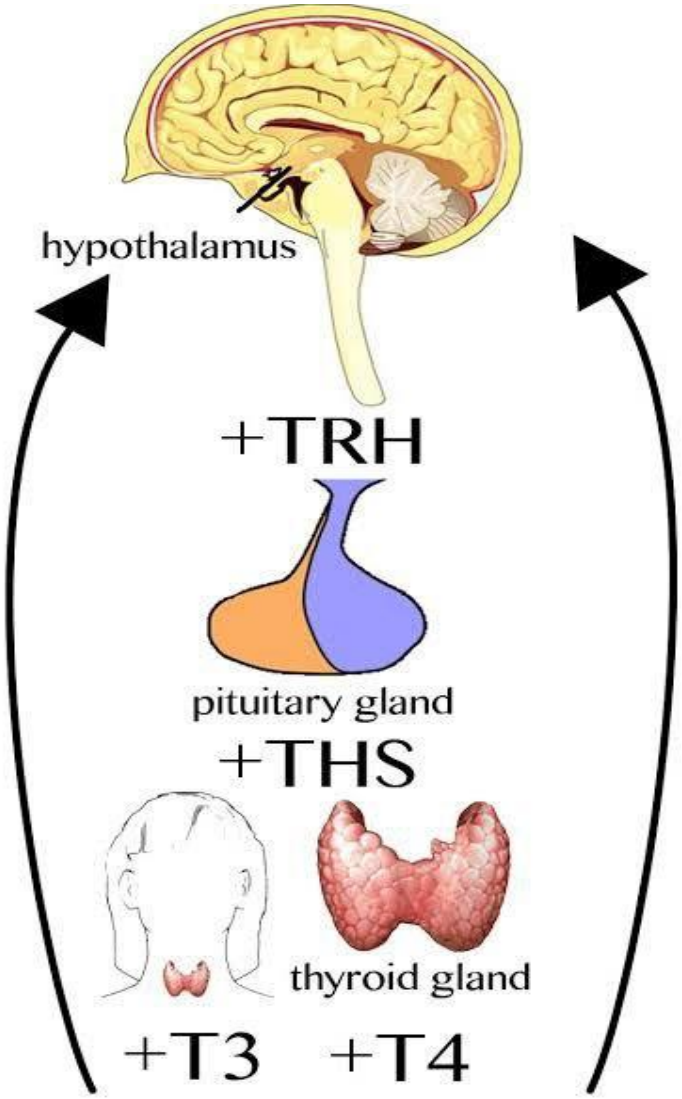
- Thyroid gland is small butterfly- shaped gland located at the front of the neck.
- The thyroid gland is a vital endocrine gland: It plays a major role in the metabolism, growth and development of the human body.
- Thyroid gland releases **triiodothyronine (T3)** and **thyroxin (T4)**, these hormones regulate metabolism, energy levels, temperature and weight.



Why to test thyroid functions ?

- To check for thyroid disorder when abnormal signs & symptoms suspicious for thyroid problem {distinguishing people with euthyroidism (normal thyroid functions) from those with hyperthyroidism (↑function) or hypothroidism (↓function)}.
- For monitoring of treatment of thyroid diseases.
- For screening of newborns for congenital hypothyroidism.

Hypothalamic – pituitary – thyroid axis



Thyroid tests can be grouped into :

Thyroid function tests

- Thyroid stimulating hormone (TSH).
- Thyroxin (T4).
- Triiodothyronine (T3).
- Free T4 (FT4).
- Free T3(FT3).

Tests for the cause of thyroid dysfunction

- Thyroid autoantibodies.
- Serum thyroglobin.
- Biopsy of thyroid gland.
- Ultrasound &isotopic thyroid scanning.

Learning outcome 1

Demonstrate methods of laboratory evaluation of thyroid hormones.

ELISA

Principle:

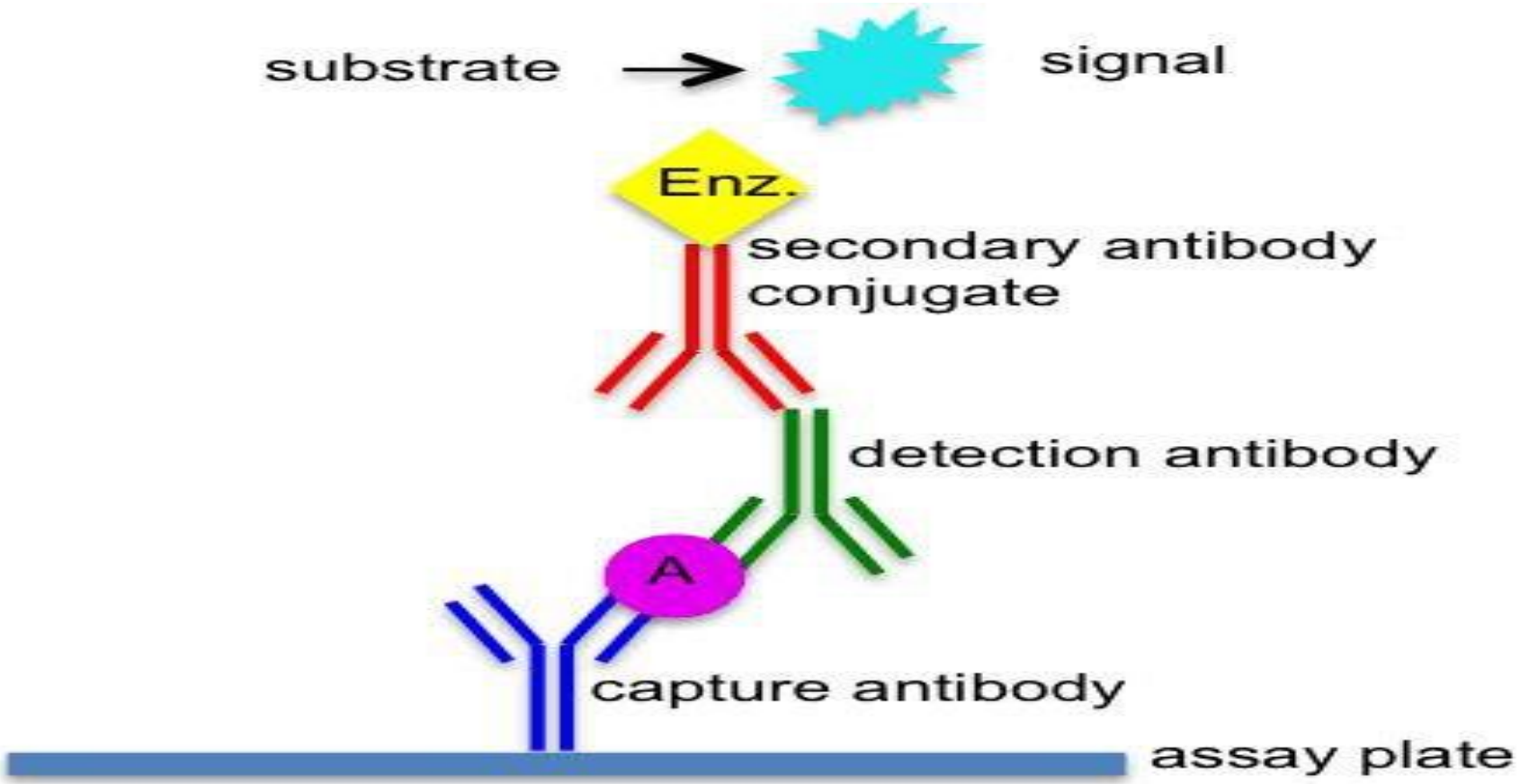
Quantitative immunological procedures which rely on **antigen- antibody reaction** and a detection system to indicate the presence and quantity of antigen binding.

TYPES OF ELISA

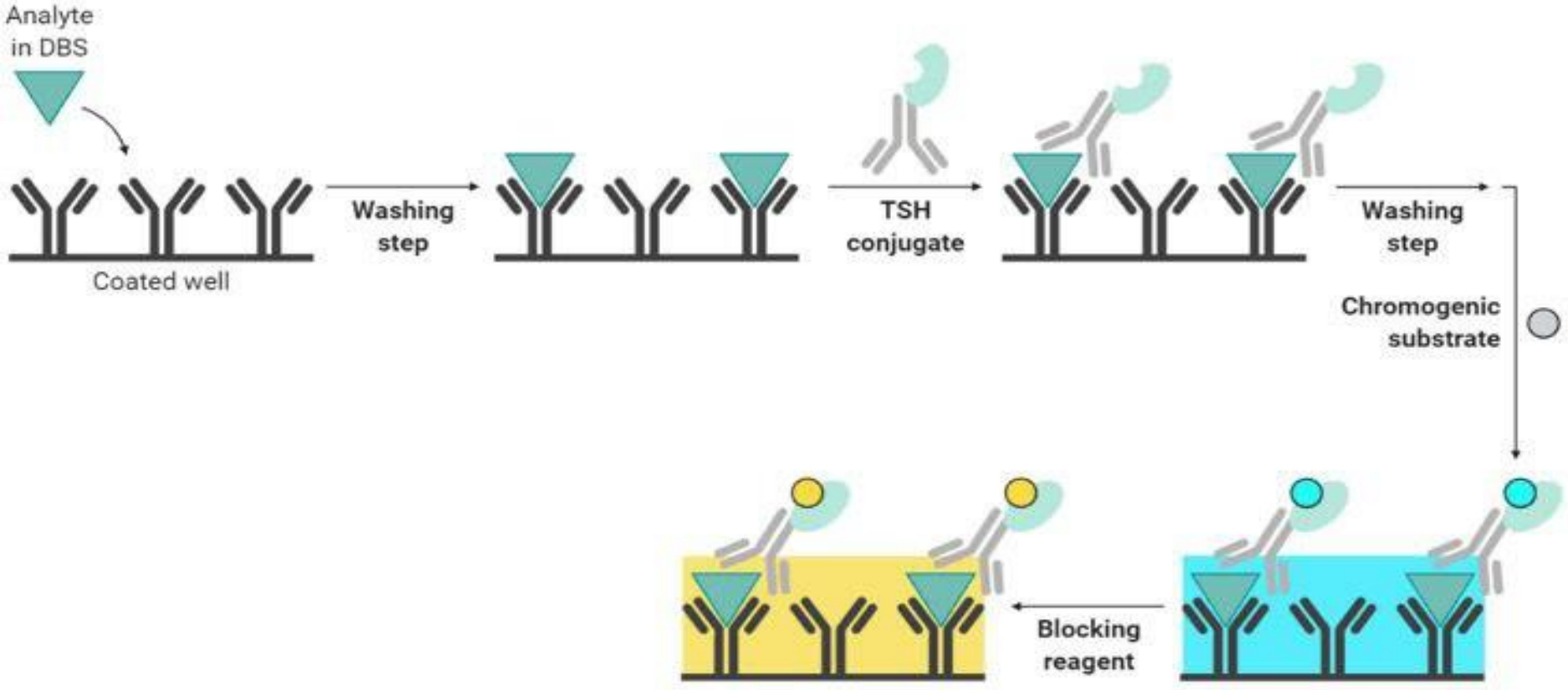
There are four major types of ELISA:

- Direct ELISA (antigen-coated plate → screening antibody).
- Indirect ELISA → (antigen-coated plate screening antigen/antibody).
- Sandwich ELISA →(antibody-coated plate screening antigen).
- Competitive ELISA→ (screening antibody).

Principle of sandwich ELISA



Principle of sandwich ELISA



Sandwich ELISA STEP BY STEP

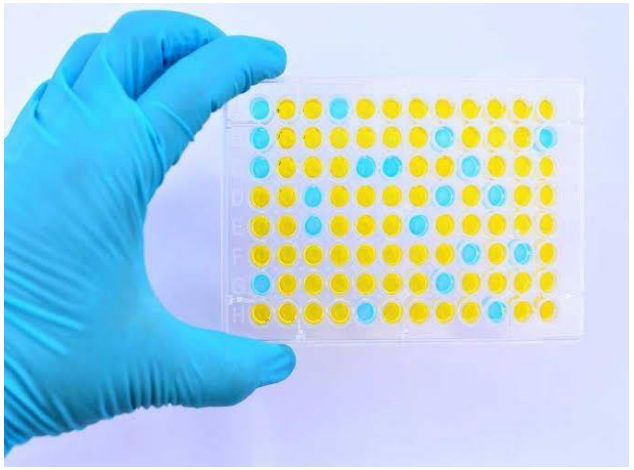
ELISA kit components

ELISA kit components

- ✓ 96- well plate
- ✓ Sample diluent
- ✓ Standard solution
- ✓ Detection antibody
- ✓ Enzyme- conjugated antibody
- ✓ Wash solution
- ✓ Chromogenic substrate
- ✓ Stop solution

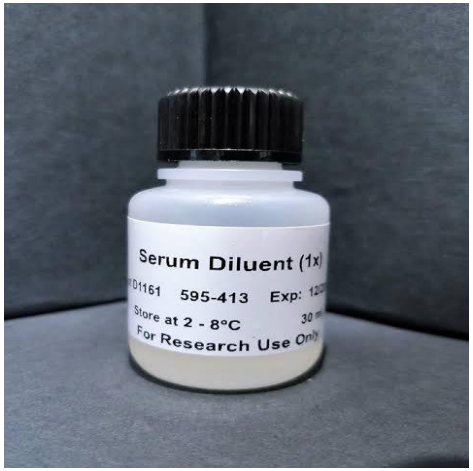
Steps of ELISA

- **Sample diluent** is added to the sample and mixed before putting them into the coated plates.



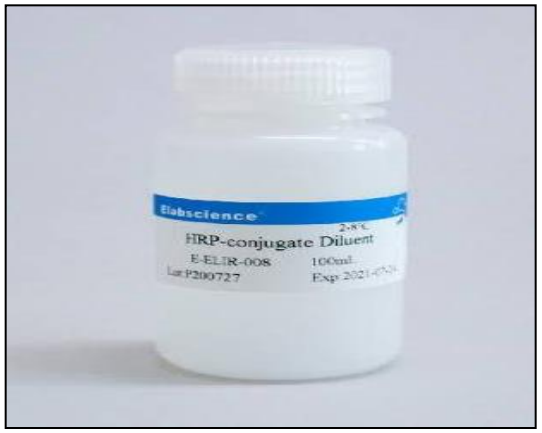
- The antibodies in coated plates (capture antibody) will bind to its antigen in sample (TSH).

- **Washing buffer** is used to remove unbounded materials.



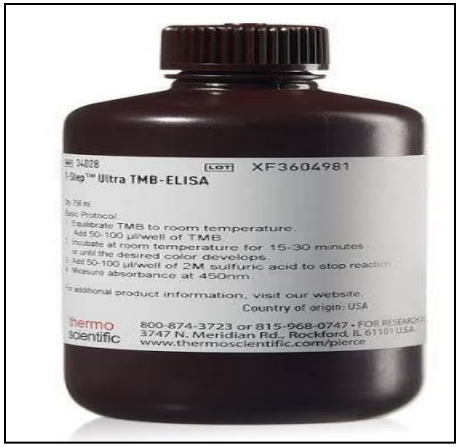
Steps of ELISA

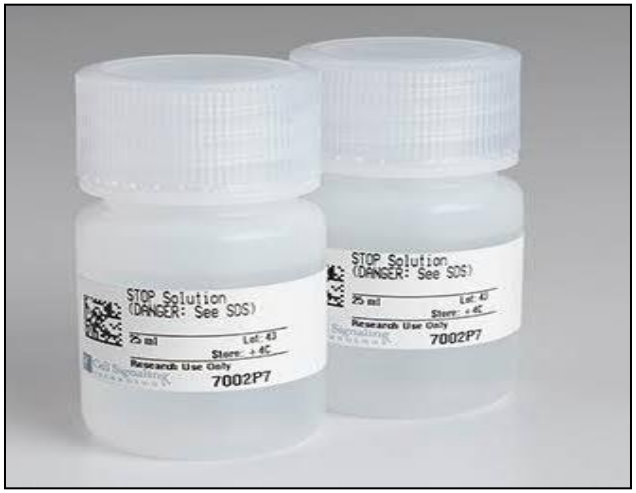
- Incubation with detection antibody.
- Wash.
- Incubation with enzyme- conjugated 2ry antibody.
- Wash.



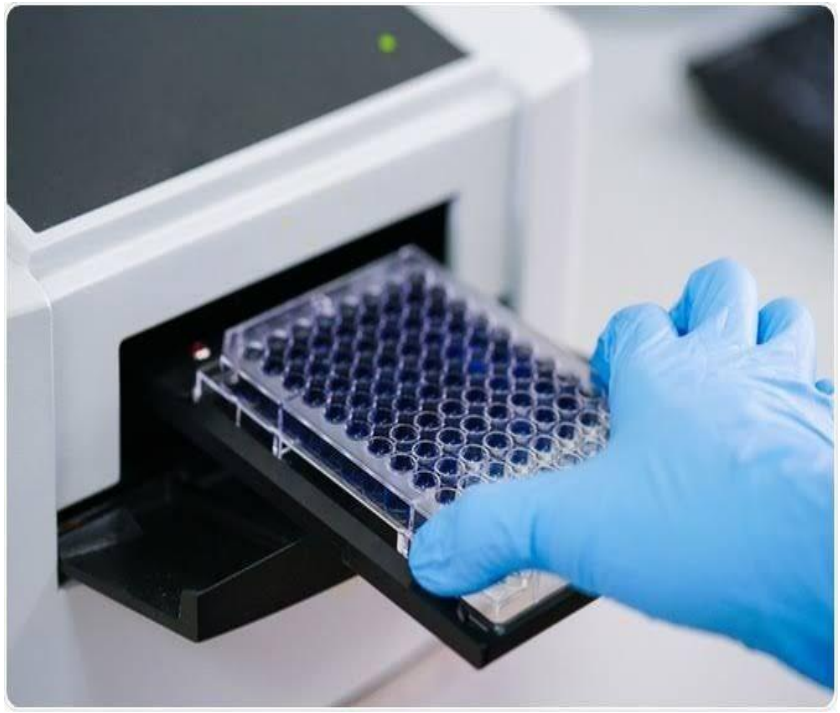
Steps of ELISA

- Incubation with substrate leading to color development.
- **Stop solution** is added to stop color development.
- Reading results via ELISA reader using standard curve.





ELISA reader



ELISA reader

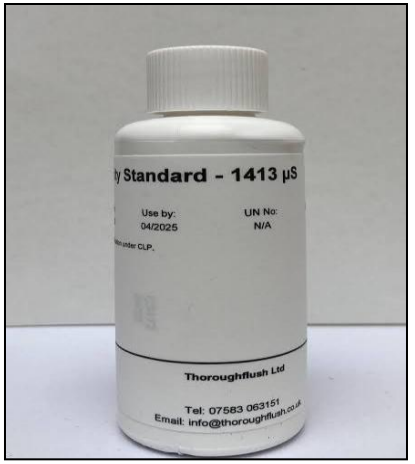
- ✓ Measure the **absorbance** of samples and standard at 450nm.
- ✓ The **concentration** of the target hormone is determined by comparing the absorbance of the samples to the standard curve.
- ✓ The higher the concentration of the target hormone in the sample the higher the degree of the color hence the higher the absorbance.

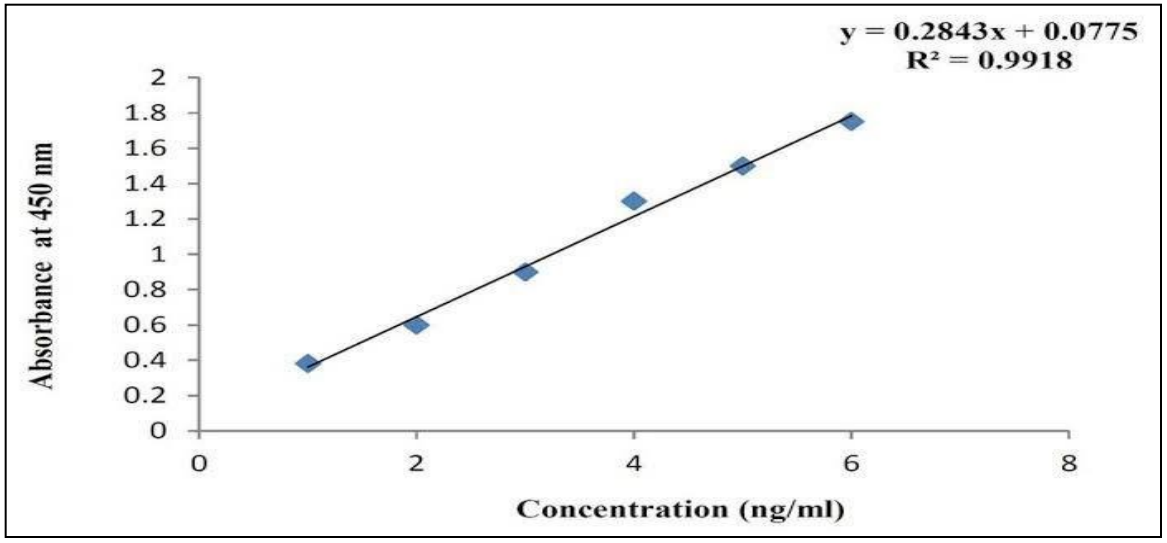
↑ concentration → ↑ degree of color → ↑absorbance

Standard curve of ELISA

❑ Standards

Prepared solutions with known concentrations used to construct a standard curve (calibration curve) for quantitative measurements of the analyte.

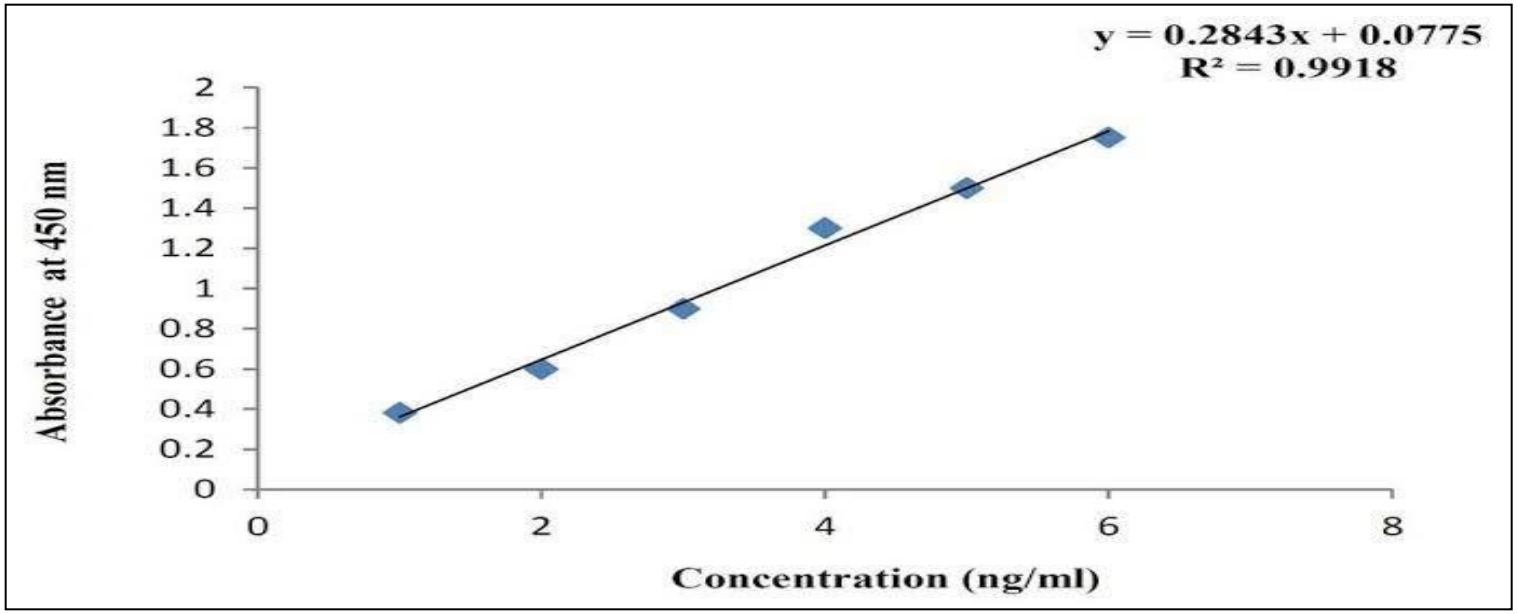




Standard curve of ELISA

Standard curve of ELISA is prepared by blotting standard concentrations on X- axis and the absorbance on Y- axis.

By using this curve, the unknown concentration of sample can be determined.



Learning outcome 2

Interpretation of the results of laboratory tests of thyroid hormones.

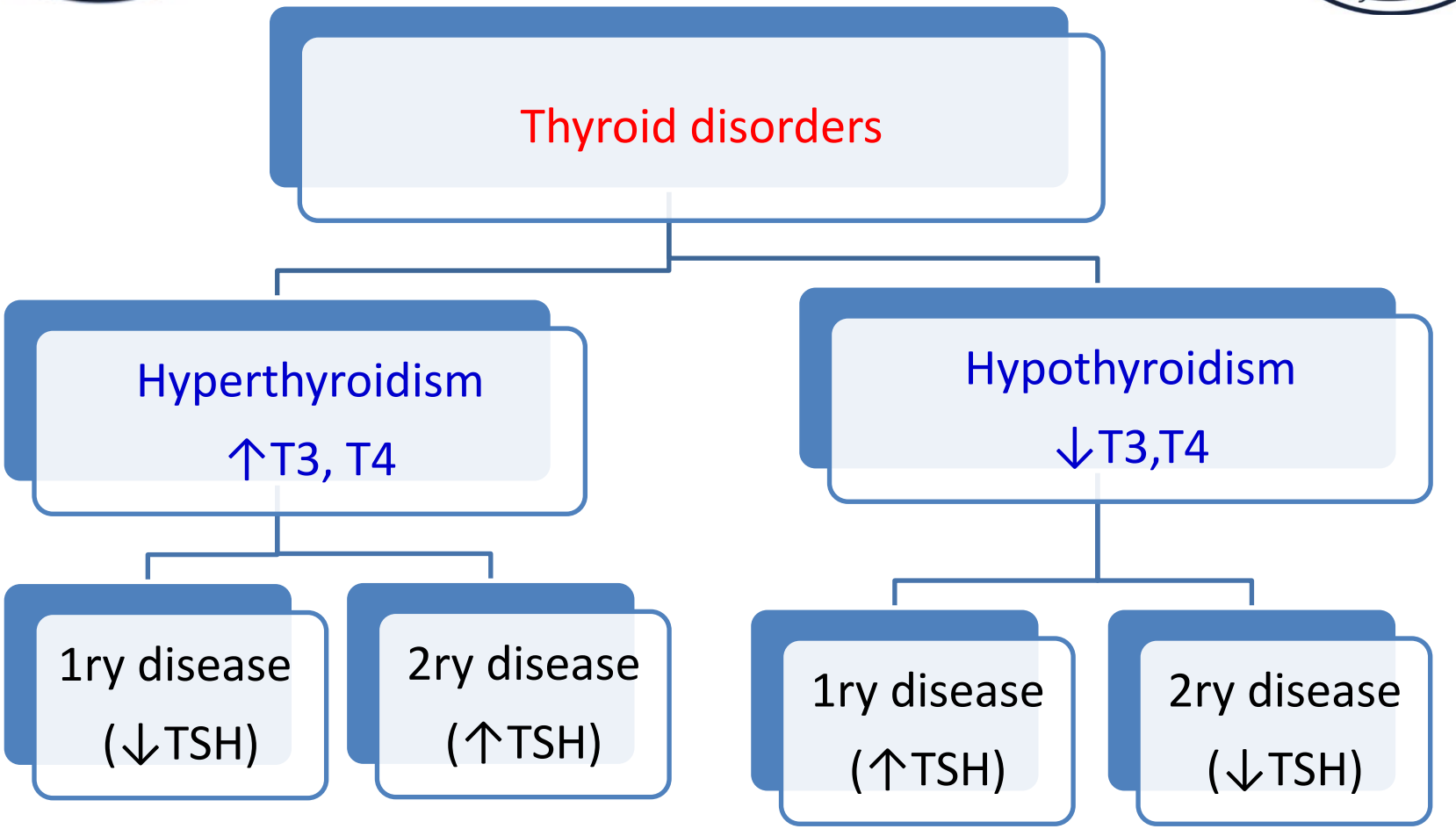
↑↑hyperthyroidism

✓ Euthyroidism

↓↓hypothyroidism

Normal values

TSH	• 0.5 - 5 mU/L
T4	• 65 -150 nmol/L
T3	• 1.8 - 3 nmol/L
FT4	• 10 -23 pmol/L
FT3	• 3 -7 pmol/L


Thyroid disorders
Hyperthyroidism
↑T3, T4
Hypothyroidism
↓T3,T4
1ry disease
(↓TSH)
2ry disease
(↑TSH)
1ry disease
(↑TSH)
2ry disease
(↓TSH)

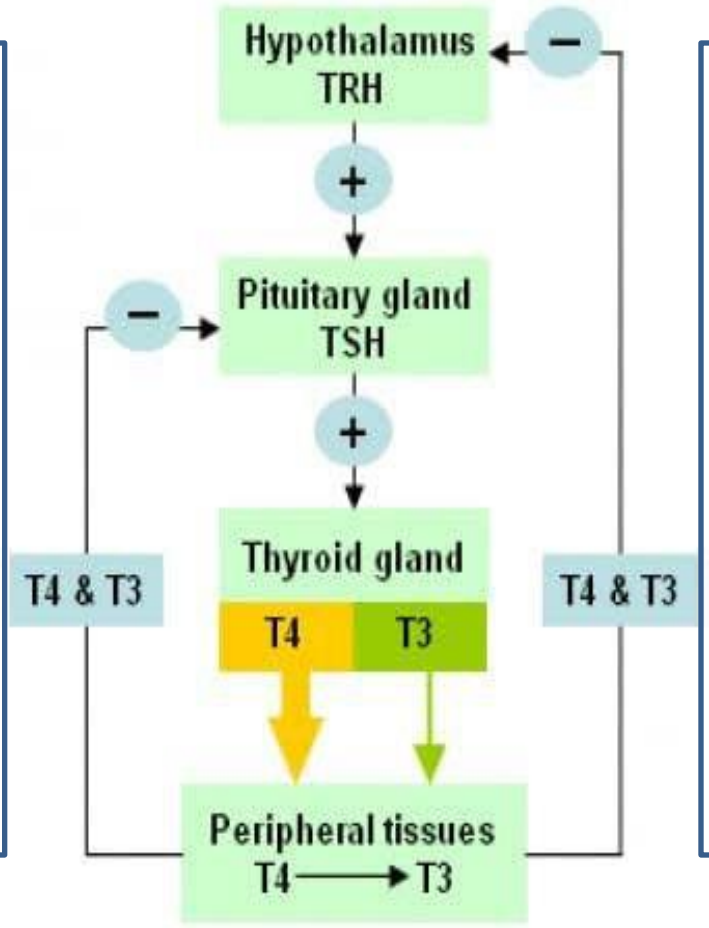
Primary thyroid disorders

(Primary = the cause of the disorder is a problem in **thyroid gland** itself)

In 1ry hyperthyroidism

when there is a problem in thyroid gland leads to increase its secretion of T3, T4 this leads to inhibition of pituitary secretion of TSH by negative feedback.

↑ T3, T4 →↓TSH



In 1ry hypothyroidism

When there is a problem in thyroid gland leads to decrease its secretion of T3, T4 this leads to stimulate pituitary gland to increase TSH secretion.

↓T3,T4 →↑ TSH

Causes of 1ry thyroid disorders

1ry hyperthyroidism

- Graves' disease
- Toxic multi-nodular goiter
- Thyroid tumor

1ry hypothyroidism

- Congenital
- Autoimmune (Hashimoto thyroiditis)
- Surgery or radiation

Secondary thyroid disorders

(Secondary = the cause of the disorder is **not** in thyroid gland itself but in either **pituitary gland** or **hypothalamus**)

- **Secondary hypothyroidism:**

  when there is defect in hypothalamus or pituitary gland

  ↓TSH → ↓T3, T4

- **Secondary hyperthyroidism:**

  due to TSH secreting tumor (very rare).

  ↑TSH →↑T3, T4

Interpretation summery

Condition	TSH	T4	T3
Euthyroidism	**Normal**	**Normal**	**Normal**
1ry hypothyroidism	↑	↓	↓
1ry hyperthyroidism	↓	↑	↑
2ry hypothyroidism	↓	↓	↓
2ry hyperthyroidism	↑	↑	↑

references

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Thank You