T4-CHECK-1

Quantitative determination of Thyroxin (Total T4) in plasma or serum samples for the detection of hyperthyroid

FOR EASY READER® AND EASY READER+® USE ONLY

Ref. 73091

I. PRINCIPLE

The Thyroid hormones, thyroxine (3, 5, 3'5' – tetraiodothyronine or T4) and liothyronine (3, 5, 3'-triiodothyronine or T3) have a great effect on metabolic activity. Although T3 is approximately three times more potent than T4, on a molar basis, the normal level of T4 in the serum is approximately 58 times that of T3. Since T4 can be measured more easily and with greater accuracy than T3, the determination of total T4 has become the primary chemical indicator of altered thyroid function.

The release of T4 and T3 from the thyroid gland is markedly influenced by the pituitary thyroid-stimulating hormone (TSH) which in turn is influenced by the hypothalamic thyrotropin-releasing hormone (TRH). Normally, increased blood levels of T4 and T3 act to decrease the amount of TSH secreted, thereby inhibiting the production and release of T4 and T3. Decreased blood levels of T4 and T3 produce the opposite effect, leading to increased production and secretion of T4 and T3. In this manner a normal circulating thyroid hormone balance is maintained. Most of the circulating T4 and T3 in the blood is bound to serum proteins, I; e., thyroxine-binding globulin (TBG), thyroxine –binding prealbumin (TBPA), and albumin. A small fraction of T4 (0.05%) is free. This free thyroxine is considered to be the metabolically active form in its effect on target tissue. The free fraction of T4 is influenced not only by the total circulating T4, but also by the concentration of thyroxine-binding proteins in the blood.

The T4-CHECK-1 is a rapid quantitative assay for the detection of Total T4 in serum or plasma to be used as a screening test for the detection of hyperthyroid The method employs a unique combination of monoclonal dye conjugate and T4 antigen coated on solid phase to identify T4 in the test samples with a high degree of specificity.

As the sample flows through the absorbent device, the labelled antibody-dye conjugate binds competitively to the T4 contained in sample and to the T4 coated in the reaction zone (T). The colour intensity of the band appearing in the test zone (T) is inversely proportional to the concentration of T4 in the sample. The mixture continues flowing through the absorbent device past the reactive zone (T) and control zone (C). Unbound conjugate binds to the reagents in the control zone (C), producing a pink colour band and demonstrating that the reagents are functioning correctly.

II- T4-CHECK-1 KIT COMPONENTS

Each kit contains everything needed to perform 10 or 20 tests.

- T4-CHECK-1 test devices :	10	20
- Disposable plastic pipettes :	10	20
- Diluent in a dropper bottle :	2.5mL	5 mL
- Instructions leaflet	1	1

- Controls (Optional):

Positive control (ref. V7300) and Negative control (ref. V7301): a freeze-dried preparation of a non-infectious compound in diluted human serum tested and found negative for anti-HIV, anti-HCV and HBs antigen, containing 0.05 % sodium azide is optionally available as a positive and negative control (1x 0.25 mL). The concentration range is indicated on the vial label.

III- STORAGE AND STABILITY

1- All T4-CHECK-1 kit components should be stored at room temperature (+4°C to +30°C) in the sealed pouch.

2- ***Do not freeze the test kit.***

3- The T4-CHECK-1 kit is stable until the expiry date stated on the package label.

IV- PRECAUTIONS

1- This test is designed for *in vitro* diagnostic use and professional use only.

2- Handle all specimens as if they contained infectious agents. When the assay procedure is completed, dispose of specimens carefully after autoclaving them for at least one hour. Alternatively, they can be treated with 0.5% to 1% solution of sodium hypochlorite for one hour before disposal.

3- Wear protective clothing such as laboratory coats and disposable gloves while assaying samples.

4- Do not eat, drink or smoke in the area where specimens and kit reagents are handled.

5- Avoid any contact between hands and eyes or nose during specimen collection and testing.

6- Do not use beyond the expiration date which appears on the package label.

7- Do not use a test from a damaged protective wrapper.

8- **IMPORTANT**: For better results, the test should be performed at an ambient temperature of +20°C as the minimum up to +30°C as the maximum. When the ambient temperature is +25°C or below, the countdown mode (internal incubation) should be preferred while the immediate mode (external incubation) should be used in case of ambient temperature over +25°C.

V- SPECIMEN COLLECTION AND PREPARATION

1- T4-CHECK-1 test is performed on human serum or plasma.

2- Patients samples are best performed if tested immediately.

3- Specimens containing precipitate may give inconsistent test results. Such specimens should be clarified prior to assaying.

4- Specimens should be refrigerated immediately at +2°C to +8°C following collection up to 3 days. If the testing within 3 days is not possible, the specimens should be frozen (-20°C). If specimens have to be shipped, they should be packed in compliance with regulations covering the transportation of etiologic agents.

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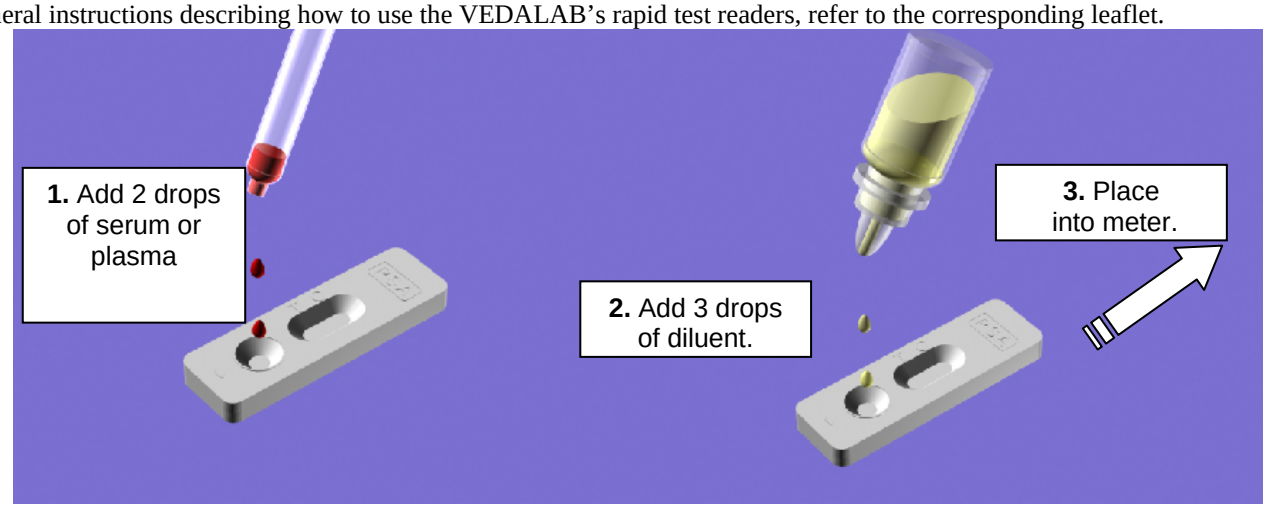
VI. ASSAY PROCEDURE

Follow the below instructions or refer to the picture n°1.

IMPORTANT: Switch the reader on and allow it to warm up for at least 30 minutes before performing any measurements.

1- Allow samples and T4-CHECK-1 test devices to come to room temperature prior to testing.
2- Remove the reaction device from its protective wrapper by tearing along the split.
3- Label device with the patient's name or control number.
4- Fill the serum dropper with specimens (serum or plasma) and by holding it vertically, dispense 2 drops (50 µL) into sample well.
5- Add exactly 3 drops (100 µL) of diluent into the sample well.
6- Read the result **(in µg/dL)** after 15 minutes either using the immediate or countdown reading mode (see the corresponding leaflet).

For general instructions describing how to use the VEDALAB's rapid test readers, refer to the corresponding leaflet.



Picture n° 1

VII. PERFORMANCES CHARACTERISTICS

a) Linearity

The measuring range is 0.6 - 15µg/dL.
For the T4 concentration lower than 0.6µg/dL, the result will be shown as "<0.6µg/dL".
For the T4 concentration higher than 15µg/dL, the result will be shown as ">15µg/dL".
For the sample having a T4 concentration exceeding 15µg/dL, dilute with saline and repeat the assay as per instructions (Part VI).
The obtained result should then be corrected using the dilution factors.

b) Accuracy

A study has been performed using serum samples pre-assayed on the Beckman Modular Analyser having a value range of 0.7 to 19.48µg/dL. Optical densities expressed as a function of T4 concentration are described by following polynominal formulation.

$$Y = 561.16x^2 + 62.80x + 2.25$$

The results show a good correlation (r>0.98) of Easy Reader and T4-CHECK-1 versus Beckman Modular Analyser.

c) Sensitivity

The sensitivity of T4-CHECK-1 is 0.6µg/dL.
Lower T4 concentrations will be shown as "<0.6µg/dL".

d) Precision

A panel of 62 serum samples pre-assayed, using the Beckmann Modular Analyser has been measured using T4-check-1 quantitative test and Easy Reader Analyser.
The results show a good coefficient of correlation of 91.1%.

e) Hook effect

The T4-CHECK-1 quantitative test is a competitive assay showing an OD decrease for increasing concentration of T4.
Therefore, there is no possibility of a hook effect in this assay.

f) Intra-assay reproducibility

The intra-assay reproducibility was evaluated by running 25 replicates of three serum samples having a T4 concentration of 2.6, 5.0 and 10.5µg/dL. The obtained CVs (coefficient of correlation) were 21.6%, 17.2% and 15.3% respectively.

g) Inter assay reproducibility
Between lots reproducibility was determined by using two specimens containing 5.2 and 12.0 µg/dL of T4, tested in 3 independent assays with three different lots of reaction device. The obtained coefficients of correlation (CV) were respectively 7.4% and 12.8%.

h) Cross reactivity
The following substances were tested for cross-reactivity (Data supplied by the anti T4 antibody manufacturer).

Substances	Cross reactivity %
Thyroxin (T4)	100
L- Triiodothyronine	0.78
D- Triiodothyronine	0.68

i) References values
Normal total T4 values are ranging from 4 to 11 µg/dL from litterature.
However it is strongly recommended that each laboratory determines its own normal range values.

j) Conversion factor
The T4 concentration could be expressed either in µg/dL or in nmol/L.
The conversion factor is:

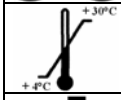
- nmol/L = concentration in µg/dL X 12.88.

VIII. LIMITATIONS

1- As for any diagnostic procedure, the physician should evaluate the data obtained using this kit in the light of the other clinical information available.
2- This format of test is to be only used with VEDALAB rapid test readers (EASY READER® or EASY READER+®).
3- If the reading time (15 minutes) is not strictly respected, wrong results will be obtained.
4- This format of test should not be used for visual reading.
5- As for any diagnostic method or for any measurements through analysers, there is a variability of the obtained result. Therefore, a confidence range of +/- 25% should be considered for the final value and for the clinical significance of the result.
6- It is recommended that each laboratory establish its own references ranges based on representative patient population in order to test the validity of the supplied data. Therefore, the data given should only be intended as orientational guidelines.
7- For better results, it is recommended to strictly follow the proceeding temperature recommendations as well as to warm up the reader for 30 minutes before starting measurements.

IX. BIBILIOGRAPHY

1. **Oppenheimer JH:** Role of plasma proteins in the binding, distribution and metabolism of the thyroid hormones. New Engl. J. med. 278: 1162 (1968).
2. **Hamburger JI :** Perspectives on Diagnostic techniques. In thyroid today: an endocrine update, SH Inger, ed. MEDCOM Learning systems, Deerfield, IL, (1973).
3. **Tietz NW:** Clinical guide to laboratory tests. W.B. Saunders company, Philadelphia, p.478 (1983).

	Read the instructions before use		For *in vitro* diagnostic use
	Temperature limitations		Do not reuse
	Manufacturer		

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