T4 QUANTI CARD

Fluorescence immunoassay (antigen-antibody) for
Quantitative measurement of Total Thyroxine (T4) in Human Serum/ Plasma

INTRODUCTION

Thyroxine (T4) is one of the main thyroid hormones, and has a molecular weight of 777 Da. T4 enable to estimulate synthesis and energy metabolism, to increase basal metabolic rate and oxygen consumption, and to stimulate growth and development. In blood, 99.97% of T4 binds to thyroxine - binding globulin (TBG) and thyroxine - binding prealbumin (TBPA). T4 is one of the indicators of diagnostics and efficacy evaluation of hyperthyroidism and hypothyroidism.

INTENDED USE

T4 Quanti Card is a sensitive immunoassay for the quantitative determination of T4 (Thyroxine) in human serum/ plasma with iQuant Analyzer.

PRINCIPLE (ANTIGEN-ANTIBODY REACTION)

T4 Quanti Card is based on principle of competitive immunoassay. The T4 from the sample is first released/extracted. Extracted T4 from test sample and T4 biotin conjugate competes for the limited number of binding sites of specific antibodies fluorochrome conjugate. As a result higher concentration of T4 produces a lower fluorescence signal and vice versa. The signal is interpreted and displayed on iQuant Analyzer in form of results.

KIT PRESENTATION & MATERIALS PROVIDED

T4 Quanti Card Test kit contains following components to perform the assay:

S. No.	Component	25 Test Pack	50 Test Pack
1.	T4 Quanti Card	25 Nos.	50 Nos.
2.	Releasing Buffer	1 Vial	2 Vials
3.	Conjugate-A	1 Vial	2 Vials
5.	Instruction Manual	1 No.	1 No.

MATERIAL REQUIRED, BUT NOT PROVIDED

iQuant Analyzer
Micropipette & Microtips
Stop Watch

STORAGE AND STABILITY

T4 Quanti Card should be stored at 2-8ºC in the coolest & driest area available. Expiry date on the kit indicates the date beyond which kit should not be used. The kit should not be frozen & must be protected from exposure to humidity and direct sunlight.

DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical cartridges and their packing. They are explained in more detail in the British and European Standard EN ISO 15223-1:2016.

- Manufactured By
- No. of tests
- LOT — Lot Number / Batch Number
- Manufacturing Date
- Expiry Date
- Single use only
- Do not use if package is damaged
- IVD — In vitro diagnostic medical device
- See Instruction for use
- 4°C / 30°C — Temperature Limitation
- Caution, see instruction for use
- REF — Catalogue Number
- Keep away from sunlight

WARNING FOR USERS

⚠ **CAUTION:** ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for in vitro diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. All materials used in the assay and samples should be decontaminated in suitable disinfectant solution for 30-60 min. before disposal or by autoclaving at 121ºC at 15psi for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures and guidelines.
7. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
8. Conjugate-A contains Sodium Azide as a preservative. If these material are to be disposed off through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds. In addition, consult the manual guideline "Safety Management No. CDC-22", Decontamination of Laboratory Sink Drains to remove Azide salts" (Centre for Disease Control, Atlanta, Georgia, April 30, 1976).

PRECAUTIONS

In order to obtain reproducible results, the following instructions must be followed:

1. Use disposable gloves while handling potentially infectious samples and performing the assay. Wash hands thoroughly afterwards.
2. Do not use the kit beyond the expiry date.
3. Do not mix reagents from different batches.
4. Do not open the foil pouch until it attains room temperature.
5. Do not re-use the test device.
6. Use separate pipette tips for each sample in order to avoid cross-contamination of samples which could cause erroneous results.
7. Follow the given test procedure and storage instructions strictly to get accurate results.

Important Note: T4 Quanti Card is only operational in conjuction with iQuant Analyzer.

SAMPLE / SPECIMEN COLLECTION AND STORAGE

1. Collect the whole blood in a clean container (containing EDTA, sodium floride or heparin) by venipuncture. Fresh samples are preferred for testing as they perform best when tested immediately after collection.
2. If specimens cannot be tested immediately, they should be refrigerated at 2-8ºC for 3 days.
3. If testing within 3 days is not possible, specimen should be frozen at -20ºC or below for 3 months.
4. Repeated freezing and thawing of the specimen should be avoided.
5. Specimens containing precipitate or particulate matter may yield inconsistent test results. Such specimens must be centrifuged and the clear supernatant should only be used for testing.
6. The use of hemolytic, lipaemic, icteric or bacterially contaminated specimens should be avoided as it may lead to erroneous results.

BEFORE YOU START

1. Switch on the iHeating block before starting the test procedure and make sure it should attain the factory preset (50°C) temperature required for testing. As the iHeating block is switched ON, its "RED" LED indicator will glow and upon attaining the desired temperature its "GREEN" LED will glow. It may take approximately 15-20 min to achieve the factory preset temperature. The "RED" LED indicator may go ON/OFF as per heating requirement. However "GREEN" LED will glow continuously. Once its "GREEN" LED is ON, the instrument is ready for use.
2. Plug in the iQuant Analyzer. Press the Power button of the iQuant analyzer, it will take approximately 1 minute for its self-checking and when the test screen will come, one can start the test procedure.
3. Plug in the iVortexer.
4. Bring the complete test kit and samples to be tested to room temperature (RT) prior to testing. **RT 20-30°C**

TEST PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the instructions given in this instruction for use and with adherence to good laboratory practice.

Step A: T4 Releasing Step:

I) Take **50µl of serum sample** in a fresh micro tube provided in the kit and add **50µl of Releasing Buffer** followed by addition of **50 µl of Conjugate A**. Mix well by vortexing for 15 sec using iVortexer and incubate for 10 minutes by placing it in the pre-heated iHeating block. **Use separate tips for each pipetting step.**

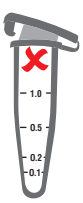


Fig. (a)

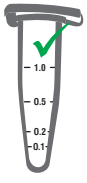
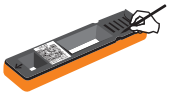


Fig. (b)

Note: Close the lid of the microtube properly by pressing it twice to ensure proper locking as shown in fig. (b) and it should be followed for each step.

Step B: Assay Procedure for running the test:

I) Remove the test cartridge from foil pouch prior to use and place it on a flat and dry surface. The test should be performed immediately after removing the test cartridge from foil pouch.



ii) Label the test cartridge with patient's name or identification number. **DO NOT write on QR Code**.

iii) After incubation is complete, load the **75µl of** the mixed solution to the **sample well** of the cartridge. **Care should be taken to avoid any spillage on the QR Code.**

iv) Allow the reaction to occur for **20 minutes** at room temperature (20-30°C). In the meantime enter the patient's details in the iQuant analyzer testing window and select the T4 test from the pop down menu in the testing window of the i Quant analyzer.

v) Insert the test cartridge after **20 minutes** into the iQuant analyzer with arrow (←) marked side on the top of the cartridge facing towards the iQuant analyzer.

vi) Press the "RUN" icon on the i Quant analyzer test window.

vii) Note down the T4 value displayed on the result window of the iQuant analyzer.

viii) Discard the T4 Quanti Card immediately after reading the results at **20 minute** considering it to be potentially infectious.

MEASURING RANGE

The measuring range of T4 Quanti card is 1-25 µg/dl.

Detection Limit: 1 µg/dl

Each laboratory should establish its own range of normal value. The values given below are only indicative.

Distribution of normal values ranges from 4.4 to 10.8 µg/dl in Male and from 4.8 to 11.6 µg/dl in Female.

PERFORMANCE CHARACTERISTICS OF T4 Quanti Card

1. Precision

Intra-Assay: Within-run and between-run precision have been determined by testing 10 replicates of 5 different samples with T4 concentration (2µg/dl, 5µg/dl, 10µg/dl, 20µg/dl & 24µg/dl respectively) on the same lot on same day. The C.V (%) is ≤ 5%.

Inter-Assay: The inter-assays were performed with 10 replicates of 5 different samples T4 concentration (2µg/dl, 5µg/dl, 10µg/dl, 20µg/dl & 24µg/dl respectively) of three different lots on 10 sequential days. The C.V (%) is ≤10%.

2. Accuracy

The T4 concentration of 542 clinical specimen were quantified independently with T4 Quanti Card and commercially available certified kit. The following results were obtained:

Slope : 0.9935
Y-Intercept : 0.0024
R^2 : 0.995

3. Sensitivity

The analytical sensitivity of T4 Quanti Card is 1.0 µg/dl.

4. Specificity

There was no significant interference with the T4 measurement was observed when other biomolecules; Tetraiodothyroacetic acid (300 nmoL/lt), l-Triiodothyronine (500 nmoL/lt), d-Triiodothyronine (500 nmoL/lt), l-Tyrosine (5000 nMol/lt) d-Tyrosine (5000 nmoL/lt) were added to the test specimen with much higher level in normal blood.

LIMITATIONS AND INTERFERENCES

1. The test procedure, precautions and interpretation of results for this test must be strictly followed.
2. As with all diagnostic tests, the test result must always be correlated with clinical finding and laboratory data available.
3. Any modification to the above procedure and / or use of other reagents will invalidate the test procedure.
4. Technical / procedural errors as well as the presence of additional substances in blood samples may interfere with product performance and may cause erroneous results.
5. The test has been developed for testing Human serum/ plasma samples only.

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an in vitro diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. The manufacturer shall not be liable to the purchaser or third par ties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

REFERENCES

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TROUBLE SHOOTING

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. Unexpected low test result	a) Incubation period of Releasing step less than 10 minutes.	Repeat the test with incubation period of 10 minutes.
	b) High volume of Conjugate-A used.	Use accurate volume of Conjugate-A.
	c) Reagents used were too cold & were not brought to room temperature (R.T.)	Bring the whole test kit to R.T. before testing.
2. Unexpected high test result	a) Low volume of reaction mixture applied to sample well of cartridge.	Repeat test with appropriate loading Volume.
	b) Expired test kit used.	Repeat test using a new test kit that has not passed the expiration date.
	c) Low volume of Conjugate-A used.	Use accurate volume of Conjugate-A.
	d) Conjugate-A is continuously exposed to light for more than 8 hrs.	Always recap the Conjugate-A vial after use. Store at 2-8°C.
3. Test Result do not match with other Elisa or CLIA method	a) Result on Elisa or CLIA kit can be more or less than actual value due to improper calibration of instrument.	Recheck the sample after proper calibration of instrument.
	b) Procedural error.	Check the procedure and perform test again as per kit product insert. Retest the sample on standard ELISA or CLIA kit to confirm the correct value of the sample.

For in-vitro diagnostic use only, not for medicinal use

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