DBC Reverse T_3 ELISA

DON'T LET YOUR THYROID TAKE OVER

Test Your Hormones

DBC's panel of thyroid hormone immunoassays is now more comprehensive with the **new Reverse T3 ELISA kit**

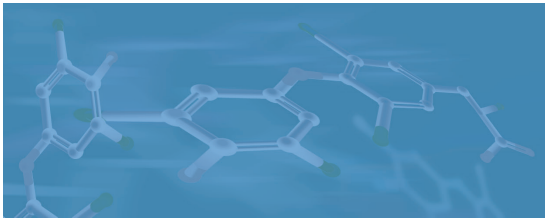
Reverse T_3 is the Best Marker of Tissue Thyroid Hormone Levels

DBC

Diagnostics Biochem Canada

INTRODUCTION

For years thyroid hormone testing has been concentrated on TSH and T4. Serum TSH and T4 levels however, do not correlate well with intracellular thyroid hormone levels.[1, 2] This recent finding counters the long-held misconception that the rate and extent of uptake of thyroid hormones into the cells occurs by simple diffusion (propelled by the concentration of the free hormones in serum).



Instead, the transport of T3 and T4 into the cells across the cellular membranes is active and requires cellular energy, which affects the T4 transporter more than the T3 transporter. Therefore, TSH and T4 serum concentrations are poor indicators of tissue thyroid levels and should not be used to diagnose if the individual is euthyroid (normal thyroid hormone concentration) at the tissue level. Moreover, high T4 levels have been negatively correlated with the conversion of T4 to T3 (the active thyroid hormone). In spite of overwhelming support for this mechanism, the misconceived "diffusion hypothesis" continues to be held by endocrinologists and primary physicians, sometimes leading to inadequate prescriptions of T4 preparations such as Synthroid and Levoxyl for restoring tissue euthyroidism.

Furthermore, the thyroid hormone status is disturbed in non-thyroid illnesses such as sepsis, surgery, myocardial infarction, starvation, and others where the prevalence of abnormalities in thyroid function tests is between 40 and 70% with consequent difficulties in interpretation of the results that leads to patient mismanagement.

What then, is the best marker of tissue thyroid hormone levels?

The answer lies in a structural isomer of T3:

Reverse T3

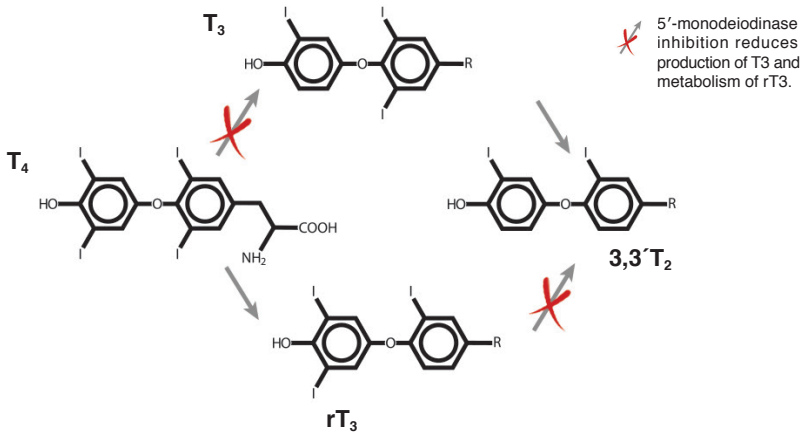
Reverse T3 (rT3), or reverse triiodothyronine (3,3′,5′-Triiodo-L-thyronine), differs from triiodothyronine (T3; 3,3′,5-Triiodo-L-thyronine) in the positions of the iodine atoms in the molecule. Additionally, the majority of circulatory rT3 is synthesized by peripheral deiodination of thyroxine (T4).

Both T3 and rT3 bind to thyroid hormone receptors; but, in difference to T3, rT3 has not been found yet to stimulate receptor metabolic activity. It does however, block receptor sites from T3 activation. The ratio of rT3 to T3 is a valuable biomarker of the metabolism and function of thyroid hormones because the process of 5′ monodeiodination that converts T4 to T3 and rT3 to 3,3′-T2 (see diagram on p.3) is inhibited in a number of non-thyroidal conditions such as fasting, anorexia nervosa, malnutrition, diabetes mellitus, stress, severe trauma or infection, hemorrhagic shock, hepatic dysfunction, pulmonary diseases and others (except renal failure and AIDS). This scenario is known as "Sick euthyroid" syndrome or "Low T3" syndrome.

An elevated ratio of rT3 over T3 is therefore indicative of "sick euthyroid" syndrome and helps to exclude a diagnosis of hypothyroidism, particularly in critically ill patients.[1-11]

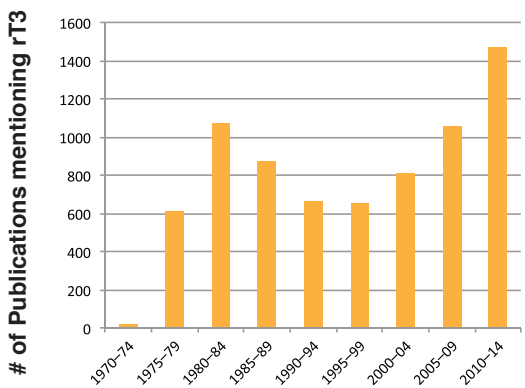
The concentration of rT3 could be high in patients on the following medications: amiodarone, dexamethasone, propylthiouracil, ipodate, propranolol, and the anesthetic halothane; on the other side, the concentration of rT3 could be low in patients on Dilantin which decreases rT3 due to its displacement from thyroxine-binding globulin and therefore generates excessive clearance of rT3.

In non-thyroidal illness the inhibition of 5′-monodeiodinase produces decreased conversion of T4 into T3 and rT3 into 3,3′-T2.



Most authors agree that measurements of rT3 are considered useful in differentiating nonthyroidal illness (high rT3) from secondary hypothyroidism (low TSH and possible low rT3) with one report in 1995 claiming poor diagnostic specificity.[12] Lately, however, the diagnostic importance of rT3 tests has been recognized and the interest on this hormone has increased dramatically.

The number of publications on rT3 have more than doubled in the last decade and have rocketed in the last few years.



Currently clinical diagnostic rT3 testing is commercially available only through expensive technologies—LC-MS/MS or RIA—which are out of the reach for many laboratories in the World.

Recognizing this, DBC developed a simple and accurate ELISA test for the determination of rT3 in serum, and plasma.

PRINCIPLE OF THE TEST

The DBC rT3 ELISA is a competitive enzyme immunoassay, where the antigen (rT3 present in calibrators, controls and patient samples) competes with a biotin-labelled antigen (rT3-Biotin conjugate) for a limited quantity of antibody coated on the microplate wells. After one hour incubation followed by the first washing, unbound materials are removed and a Streptavidin-HRP conjugate is added and incubated for 30 minutes followed by a second washing and addition of TMB, the HRP substrate. The enzymatic reaction is terminated by addition of stopping solution upon which the colour intensity—measured with a microplate reader—is inversely proportional to the concentration of rT3 in the sample. The set of kit calibrators that are run simultaneously with the samples is used to plot a calibration curve and determine the concentration of rT3 in samples and controls.

FOUR EASY STEPS

			Solution Colour in Well
1	Load calibrators, controls and samples; add biotin conjugate	Incubate 1 hour at 37°C	
2	Wash and load Streptavidin-HRP conjugate	Incubate 30 minutes at 37°C	
3	Wash and load substrate (TMB)	Incubate 15 minutes at 37°C	
4	Add stopping solution	Read in a microplate reader at 450 nm	

PERFORMANCE

SPECIFICITY (CROSS-REACTIVITY)

The quantitative evaluation of the cross-reactivity was performed using the method of Abraham.[13]

Compound	%Cross Reactivity
rT3	100
T3	< 0.001
T4	0.005
3,5-T2	0.004

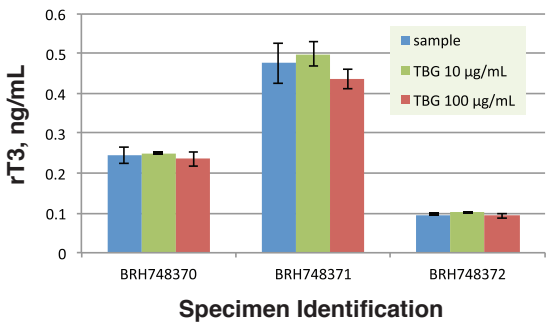
INTERFERENCES

The following substances did not show significant interference with the assay: hemoglobin up to 2 g/L, free and conjugated bilirubin up to 200 mg/L, triglycerides up to 5.5 mg/mL and Biotin up to 40 µg/mL.

Effect of TBG concentration in samples on rT3 concentration results measured with DBC's rT3 ELISA kit.

To demonstrate that DBC's rT3 ELISA blocks interference of TBG binding to rT3, TBG was added to human serum samples at concentrations of 10 and 100 µg/mL. The sample results are not affected significantly with the presence of additional TBG in the sample. TBG's reference concentration range in serum is 11–27 µg/mL.

Error bars represent the SD of the mean of two independent experiments each with two replicated measurements.



PERFORMANCE

INTRA-ASSAY PRECISION

Four serum samples were assayed 24 times each on the same calibrator curve.

Sample	Mean (ng/mL)	SD (ng/mL)	%CV
1	0.089	0.0024	2.7
2	0.250	0.020	8.0
3	0.455	0.019	4.2
4	1.018	0.140	13.8

INTER-ASSAY PRECISION

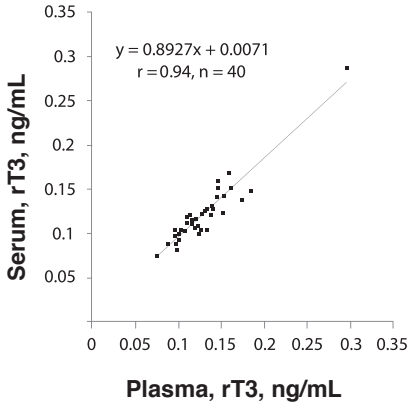
Four serum samples were assayed in 20 different tests in the period of 10 days. The results are tabulated below:

Sample	Mean (ng/mL)	SD (ng/mL)	%CV
1	0.127	0.016	12.6
2	0.304	0.038	12.5
3	0.469	0.057	12.2
4	0.847	0.083	9.8

PERFORMANCE

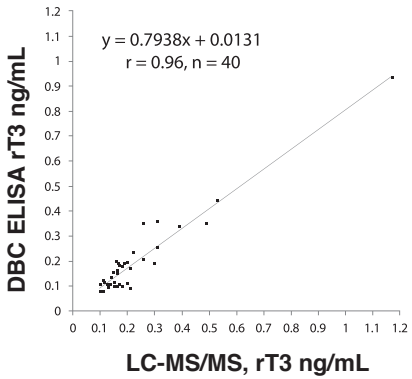
SAMPLE MATRIX COMPARISON

The option to use plasma samples was investigated using 40 matched sample pairs of each serum and plasma (di-sodium EDTA) from the same individuals. The regression study demonstrates that there is strong equivalence between matrices, therefore the type of matrix, serum or plasma, does not affect the analytical result of the test.



COMPARATIVE STUDIES

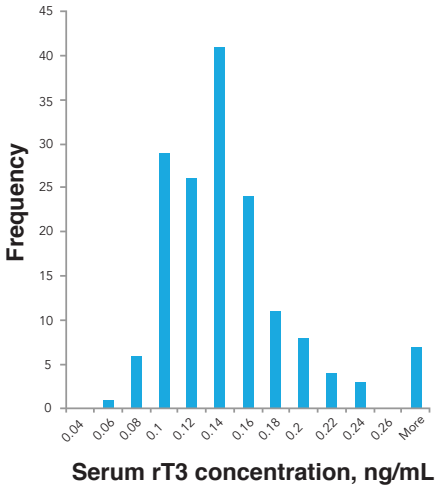
The new device was compared to a leading competing technology: LC-MS/ MS. The comparison was performed with 40 commercial human serum samples and shows that the devices produce commutable results:

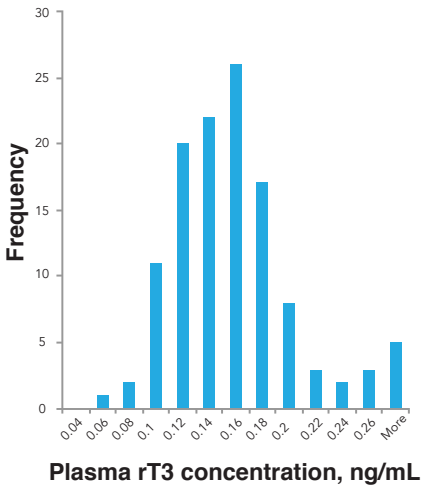


PERFORMANCE

Reference ranges calculated using a non-parametric method, using commercial samples from putatively normal individuals 20 years or older. Data in ng/mL.

Group	n	Median	95% Confidence Range	Total Range	Published Range at Mayo Clinic
Serum adults	160	0.13	0.08–0.31	0.06–0.76	0.1–0.24
Plasma adults	120	0.14	0.08–0.29	0.049–0.65	





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DBC Reverse T_3 at a glance

- DBC rT3 ELISA REF: CAN-RT3-100
- Sample: 25 µL of human serum or plasma
- No sample preparation
- Total assay time: less than 2 hours
- Number of calibrators: 6
- Number of supplied internal controls: 2
- Sensitivity: 0.005 ng/mL
- Cross-reactivity:

T3	< 0.001%
T4	0.005%
3,5-T2	0.004%

- Correlates with LC-MS/MS results
- Automatable

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