

# PROVIDER DATA SHEET

## Comprehensive Iodine-Thyroid Profile in Dried Blood Spot and Dried Urine



Thyroid dysfunction is a health issue often caused by iodine deficiency as well as iodine excess<sup>1,2</sup>. Although iodine is an essential component of the thyroid hormones thyroxine (T4) and triiodothyronine (T3), very little clinical emphasis is placed on iodine's availability for synthesis of thyroid hormones or its role in conditions of thyroid dysfunction (hypothyroidism or hyperthyroidism).

ZRT Laboratory has combined the advanced technology of iodine determination in dried urine, with that of thyroid hormone measurements in finger stick dried blood spots (DBS) to create the "Complete Iodine-Thyroid Profile". This unique Profile is designed to evaluate not only iodine's availability, but also its capacity to be utilized for thyroid hormone synthesis.

### Tests Included in the Complete Iodine-Thyroid Profile and Rationale for their Use

#### Dried Urine - Iodine

Iodine is an essential component of the thyroid hormones T4 and T3<sup>1,11</sup>. About 90% of iodine consumed from any source (e.g., diet, supplements, medication) is eliminated in urine within 24-48 hours; therefore urine is an excellent source to determine an individual's iodine status<sup>12-15</sup>. When urine iodine levels are outside optimal ranges (too low or high), thyroid hormone synthesis can be abnormal<sup>1,16-20</sup>. Therefore information about urinary iodine status could provide clues to thyroid dysfunction and the means to correct it (i.e., increase or lower iodine levels).

#### Dried Blood Spot - Thyroglobulin

Thyroid hormone synthesis begins when iodine is transported into the thyroid gland by a trans-membrane protein called the sodium iodide symporter (NIS)<sup>21</sup>. In the thyroid follicular cell, iodide (I<sup>-</sup>) interacts with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and thyroid peroxidase (TPO) to form iodine (I<sub>2</sub>) which chemically reacts with the tyrosine residues of the protein thyroglobulin, a tyrosine rich protein synthesized only in the follicular cells of the thyroid gland<sup>22,23</sup>. Iodine reacts with tyrosine residues to form monoiodotyrosine (MIT) and diiodotyrosine (DIT) conjugates, which then couple to form the precursors of T4 (DIT + DIT) and T3 (DIT + MIT). Iodine-enriched thyroglobulin is stored in the colloidal lumen for future thyroid hormone (T3/T4) synthesis. Through a thyroid-stimulating hormone (TSH)-activated response, the iodinated thyroglobulin is then translocated back into the follicular cell where it is hydrolyzed by lysosomal enzymes to release T4 and T3, which then passively diffuse from the follicular cell into the bloodstream.

Although a very high thyroglobulin level in blood is conventionally used as a marker for thyroid carcinoma<sup>22</sup>, a moderately elevated thyroglobulin in the absence of thyroid cancer is a good marker of an individual's average exposure to iodine over a period of weeks<sup>1,22,24,25</sup>. In this sense, thyroglobulin is considered a better marker of iodine exposure than urinary iodine, which can fluctuate from day to day depending on the consumption of iodine-containing salt, foods, or supplements.

Finger stick DBS have been shown to be a convenient and patient-friendly way to test for thyroglobulin as well as other thyroid hormones<sup>26</sup>.

Thyroglobulin synthesis is initiated in the thyroid follicular cell by TSH. TSH is released from the pituitary as the hypothalamus senses low thyroid (T4/T3) hormone levels in the bloodstream. When the iodine level in the thyroid follicle is low, due to chronic iodine deficiency, inhibitors of iodine uptake or organification (goitrogenic effects), thyroglobulin is poorly iodinated<sup>1</sup> and is more likely to "leak" into the bloodstream instead of being stored in the colloidal lumen for future thyroid hormone synthesis. This wasting effect results in low iodinated-thyroglobulin reserves, which is more likely to compromise thyroid hormone synthesis when iodine levels are low. When the thyroid gland is destroyed by autoimmune thyroiditis (Hashimoto's), there is also excessive release of thyroglobulin along with thyroid hormones into the bloodstream, causing a wasting effect.

Thus, excluding thyroid carcinoma, moderate elevations in blood thyroglobulin are usually an indication that the average iodine levels over the previous weeks have been insufficient for normal thyroid hormone synthesis<sup>22</sup>. Iodine prophylaxis usually results in return of blood thyroglobulin levels to normal (< 10 ng/ml), indicating an adequate iodine supply to the thyroid gland<sup>24</sup>.

#### Dried Blood Spot - Total T4, Free T4, Free T3

The blood level of total T4 provides information about the thyroid's capacity to synthesize, process and release T4 into the bloodstream. If total T4 is low, or low normal, and thyroglobulin and TSH are elevated, this would suggest that hypothyroidism is likely caused by low iodine, or goitrogen inhibition of iodine uptake or iodination of thyroglobulin<sup>1,23</sup>.

In contrast to total T4, free T4 and free T3 provide information on the peripheral bioavailability of the active thyroid hormones to tissues. Knowing the level of these hormones in the bloodstream, however, does not predict response of target cells to them, which can be altered by many other nutritional (e.g., zinc and selenium deficiencies) and hormonal (e.g., low or high cortisol, high estrogens, low progesterone) states. Normal levels of T3, T4, and TSH, but persistent symptoms of thyroid deficiency would suggest poor cellular response to T3 and a need to investigate deeper into the underlying etiology.

Although free T4 and free T3 provide useful information on the availability of these hormones to tissues, they have not been shown to be a good surrogate marker of iodine status<sup>1</sup>. When iodine levels are very low, compensatory mechanisms come into play to assure that adequate levels of T4 and T3 are available, resulting in less fluctuation in these hormone levels with iodine deficiency. The colloidal lumen of the follicular thyroid cells normally contains a large reservoir of iodinated thyroglobulin (see above) for thyroid hormone synthesis;

however, this reservoir can be drained by prolonged iodine deficiency.

In contrast to the effects of low iodine on thyroid hormone synthesis, excessive iodine can result in hypothyroidism or hyperthyroidism depending on the preexisting conditions<sup>1,16,22</sup>. Hyperthyroidism is more likely to occur in the elderly who have lived many years in a low iodine environment and have developed autonomous thyroid nodules that hyper-respond to an acute increase in iodine consumption. Hypothyroidism on the other hand, is thought to be caused by excess iodine forming iodolipids within the thyroid follicle that inhibit TSH/cAMP-activated gene transcription of the biochemical machinery (e.g. TPO, NIS, lysosomal proteases, thyroglobulin) involved in iodination of thyroglobulin and the degradation and release of T4 and T3 from the iodinated thyroglobulin. This inhibitory effect of excess iodine on thyroid hormone synthesis, resulting in lower T4 and T3 and higher TSH and thyroglobulin levels in the bloodstream, was coined the Wolff-Chaikoff effect by the authors who first described it<sup>1,22</sup>. Most people without thyroid issues escape the Wolff-Chaikoff effect within about 48 hrs, when thyroid hormone synthesis and blood levels return to normal. However, people with subclinical hypothyroidism (elevated TSH and normal T3 and T4 levels) or Hashimoto's thyroiditis, are less likely to escape from the inhibitory actions of excess iodine as long as the high iodine state persists. In most people, lowering the iodine by removing the source (food or supplements) results in return of TSH and thyroid hormone synthesis to normal. However, others with a more compromised thyroid gland may never escape the damaging effects of high iodine and progress more rapidly to a permanent clinical hypothyroid state (low T4 and elevated TSH) and require thyroid medication.

### Dried Blood Spot - TSH

When T4/T3 levels drop below a threshold, this signals the hypothalamic/pituitary axis to synthesize and release TSH into the bloodstream. TSH, via binding to TSH receptors on the surface of the thyroid follicle, activates gene transcription/translation of much of the biochemical machinery necessary for iodine uptake (NIS), iodine organification to thyroglobulin (TPO), and lysosomal lysis and release of thyroid hormones (T4 and T3) from the thyroid follicle into the bloodstream<sup>1,22</sup>. Elevated TSH and low total and free T4 are markers of iodine deficiency. Low urinary iodine, in combination with high thyroglobulin and elevated TSH, would strongly suggest that the primary cause of hypothyroidism is low iodine.

Paradoxically, high iodine from foods or supplementation can also cause elevated TSH due to lowered thyroid hormone production in the thyroid follicular cell, as outlined above. Persistently elevated TSH in the presence of high iodine can eventually lead to goiter as TSH stimulates hypertrophy of the thyroid follicular cells<sup>1,18,22</sup>. Reduction of iodine intake to WHO/CDC recommended physiological levels (150 µg/day) usually results in reversal of the goiter<sup>1,18</sup>.

## REFERENCES - For large print, linked references, visit [www.zrtlab.com](http://www.zrtlab.com)

- Zimmermann MB. Iodine deficiency. *Endocr Rev* 2009;30(4):376-408.
- Wikipedia®. Iodine. Available at: <http://en.wikipedia.org/wiki/Iodine>
- Bleichrodt N, Born MP. A meta-analysis of research on iodine and its relationship to cognitive development. In: Stanbury JB, ed. *The damaged brain of iodine deficiency*. New York, NY: Cognizant Communications, 1994:195–200.
- Vermiglio F, Lo Presti VP, Moleti M, et al. Attention deficit and hyperactivity disorders in the offspring of mothers exposed to mild-moderate iodine deficiency: a possible novel iodine deficiency disorder in developed countries. *J Clin Endocrinol Metab* 2004;89(12):6054-60.
- Miller DW. Extrathyroidal benefits of iodine. *J Am Physicians Surgeons* 2006;11(4):106-10.
- Venturi S, Donati FM, Venturi A, et al. Role of iodine in evolution and carcinogenesis of thyroid, breast, and stomach. *Adv Clin Path* 2000;4(1):11-17.
- Cann SA, van Netten JP, van Netten C. Hypothesis: Iodine, selenium and the development of breast cancer. *Cancer Causes Control* 2000;11(2):121-7.
- Smyth PP. Role of iodine in antioxidant defence in thyroid and breast disease. *Biofactors* 2003;19(3-4):121-30.
- Hopton Cann SA. Hypothesis: dietary iodine intake in the etiology of cardiovascular disease. *J Am Coll Nutr* 2006;25(1):1-11.
- Teas J, Baldeón ME, Chiriboga DE, et al. Could dietary seaweed reverse the metabolic syndrome? *Asia Pac J Clin Nutr* 2009;18(2):145-54.
- Eastman CJ, Zimmermann MB. Chapter 20. The Iodine Deficiency Disorders. 2009. Available at: <http://www.thyroidmanager.org/chapter20/chapter20.pdf>.
- Haddow JE, McClain MR, Palomaki GE, Hollowell JG. Urine iodine measurements, creatinine adjustment, and thyroid deficiency in an adult United States population. *J Clin Endocrinol Metab* 2007;92(3):1019-22.
- Rasmussen LB, Ovesen L, Christiansen E. Day-to-day and within-day variation in urinary iodine excretion. *Eur J Clin Nutr* 1999;53(5):401-7.
- Als C, Minder C, Willems D, et al. Quantification of urinary iodine: a need for revised thresholds. *Eur J Clin Nutr* 2003;57(9):1181-8.
- Soldin OP. Controversies in urinary iodine determinations. *Clin Biochem* 2002;35(8):575-9.
- Markou KB, Paraskevopoulou P, Karaiskos KS, et al. Hyperthyrotropinemia during iodide administration in normal children and in children born with neonatal transient hypothyroidism. *J Clin Endocrinol Metab* 2003;88(2):617-21.
- Vitale M, Di Matola T, D'Ascoli F, et al. Iodide excess induces apoptosis in thyroid cells through a p53-independent mechanism involving oxidative stress. *Endocrinology* 2000;141(2):598-605.

### Dried Blood Spot - TPO Antibodies

Thyroid peroxidase (TPO) is an enzyme found within the thyroid follicular cells that, in the presence of H<sub>2</sub>O<sub>2</sub>, activates iodide (transported by NIS from the bloodstream into the thyroid follicular cell) to iodine that binds the tyrosine residues on thyroglobulin. Some individuals, for reasons not well understood but associated with selenium deficiency<sup>1,22,27</sup>, develop self antibodies to TPO, causing the autoimmune condition known as Hashimoto's thyroiditis. Over time, these antibodies attack and destroy the thyroid follicular cells resulting in fibrosis and a permanent hypothyroid state as the damaged thyroid gland no longer has the capacity for thyroid hormone synthesis. During an autoimmune attack of the thyroid, often referred to as a thyroid storm, excessive amounts of thyroid hormones and iodinated thyroglobulin are released from the thyroid gland, causing hyperthyroidism and in some cases thyrotoxicosis. Individuals using excessive iodine supplementation should be aware that they could experience a more adverse reaction if they are positive for TPO antibodies. For this reason, iodine therapy at doses that exceed the WHO/CDC recommended supplement levels (150 µg/day), should be used with caution or not at all in individuals with known or suspected Hashimoto's thyroiditis<sup>1</sup>.

### Methods for Collecting and Testing Urine and Blood

**Urine** - Iodine is collected twice during the day (first morning and last night void) on filter strips and dried overnight before sending to the laboratory for testing. The iodine content of the dried filter strips is determined following extraction and measurement by the Sandell-Kolthoff method<sup>28,29</sup>. Creatinine is measured in the same urine extract by the Jaffe method<sup>30</sup>. Iodine is expressed per liter urine and mg creatinine.

**Blood** - Whole capillary blood is collected from the finger after nicking with a lancet. Blood drops are collected on a filter card and allowed to dry (minimum 3 hr) before shipping to the laboratory. Thyroid analytes are measured in extracts of the dried blood with modified commercial immunoassays (EIA).

### Advantages of Combined Dried Urine/Dried Blood Spot (DBS) Tests

- Urine and blood sample collection and shipment are simple and convenient for the patient and practitioner and can be done at home.
- Iodine in dried urine and thyroid analytes in DBS are exceptionally stable for weeks at room temperature allowing more flexibility in collection and shipment.
- Combined urinary iodine and thyroid analyte DBS tests provide more information as to the true cause of thyroid dysfunction and a more rational approach to treating it.

- Zhao J, Wang P, Shang L, et al. Endemic goiter associated with high iodine uptake. *Am J Public Health* 2000;90(10):1633-5.
- Pedersen IB, Laurberg P, Arnfred T, et al. Surveillance of disease frequency in a population by linkage to diagnostic laboratory databases. A system for monitoring the incidences of hyper- and hypothyroidism as part of the Danish iodine supplementation program. *Comput Methods Programs Biomed* 2002;67(3):209-16.
- Poncin S, Gérard AC, Boucquey M, et al. Oxidative stress in the thyroid gland: from harmlessness to hazard depending on the iodine content. *Endocrinology* 2008;149(1):424-33.
- Shen DH, Kloos RT, Mazzaferri EL, Jhian SM. Sodium iodide symporter in health and disease. *Thyroid* 2001;11(5):415-25.
- Spencer C, Petrovic I. Chapter 7. Thyroglobulin Measurement. In: Brent GA, Ed. *Thyroid Function Testing*. Springer Science, 2010:125-153.
- Risher JF, Keith S. Iodine and inorganic iodides: human health aspects. Concise international chemical assessment document #72: World Health Organization, 2009.
- Vejbjerg P, Knudsen N, Perrild H, et al. Thyroglobulin as a marker of iodine nutrition status in the general population. *Eur J Endocrinol* 2009;161(3):475-81.
- van den Briel T, West CE, Hautvast JG, et al. Serum thyroglobulin and urinary iodine concentration are the most appropriate indicators of iodine status and thyroid function under conditions of increasing iodine supply in schoolchildren in Benin. *J Nutr* 2001;131(10):2701-6.
- Missler U, Gutekunst R, Wood WG. Thyroglobulin is a more sensitive indicator of iodine deficiency than thyrotropin: development and evaluation of dry blood spot assays for thyrotropin and thyroglobulin in iodine-deficient geographical areas. *Eur J Clin Chem Clin Biochem* 1994;32(3):137-43.
- Rose NR, Bonita R, Burek CL. Iodine: an environmental trigger of thyroiditis. *Autoimmun Rev* 2002;1(1-2):97-103.
- Ohashi T, Yamaki M, Pandav C S, et al. Simple microplate method for determination of urinary iodine. *Clin Chem* 2000;46(4):529-36.
- O'Kennedy R, Bator JM, Reading C. A microassay for the determination of iodide and its application to the measurement of the iodination of proteins and the catalytic activities of iodo compounds. *Anal Biochem* 1989;179(1):138-144.
- Bartels H, Böhrner M. Micro-determination of creatinine. *Clin Chim Acta* 1971;32(1):81-5.