

BLOOD SPOT TEST SPECIFICATIONS

Total T4

Clinical Information

Thyroxine (T4) is the primary thyroid hormone circulating in the blood. Total T4 includes both free T4 and protein-bound T4, and therefore represents the thyroid gland's capacity to synthesize, process, and release T4 into the bloodstream. In contrast, free T4 and free T3 are representative of the bioavailability of active thyroid hormones to peripheral tissues. A low level of total T4 with elevated levels of thyroid-stimulating hormone (TSH) and thyroglobulin suggests hypothyroidism caused by either low iodine intake, or inhibition of iodine uptake or the iodination of thyroglobulin by goitrogens. Excessive amounts of iodine can result in either hypo- or hyperthyroidism, depending on pre-existing conditions. For example, elderly people who have lived for a long period in a low iodine environment develop autonomous thyroid nodules as a compensatory mechanism to boost thyroid hormone production. When iodine exposure is suddenly increased, these nodules hyper-respond and the result is hyperthyroidism with high T4 production. On the other hand, hypothyroidism as a result of excess iodine can occur when iodine forms iodolipids within the thyroid follicle that inhibit the iodination of thyroglobulin, depressing T4 and T3 synthesis (the Wolff-Chaikoff effect). This can usually be reversed by lowering iodine intake to less excessive amounts, so that thyroid hormone synthesis returns to normal. However, individuals with pre-existing subclinical hypothyroidism or Hashimoto's disease may progress to a permanent clinical hypothyroid state and require thyroid hormone medication. The reference range for total T4 is 5–10.8 µg/dL.

References:

Zimmermann MB. Iodine deficiency. *Endocr Rev.* 2009;30:376-408.
Wolff J, Chaikoff IL. The inhibitory action of excessive iodide upon the synthesis of diiodotyrosine and of thyroxine in the thyroid gland of the normal rat. *Endocrinology.* 1948;43:174-9.
Aoki Y, Belin RM, Clickner R, et al. Serum TSH and total T4 in the United States population and their association with participant characteristics: National Health and Nutrition Examination Survey (NHANES 1999-2002). *Thyroid.*

Assay Method: Fluoroimmunoassay

Intra-assay Precision

Intra-assay precision was determined by choosing three samples spanning the reference range, and analyzing them multiple times within the same run. Results are shown below:

Mean Total T4 Concentration (µg/dL)	Standard Deviation	Coefficient of Variation (C.V. %)
5.9	0.35	6.1
7.8	0.54	7.0
9.4	0.50	5.4

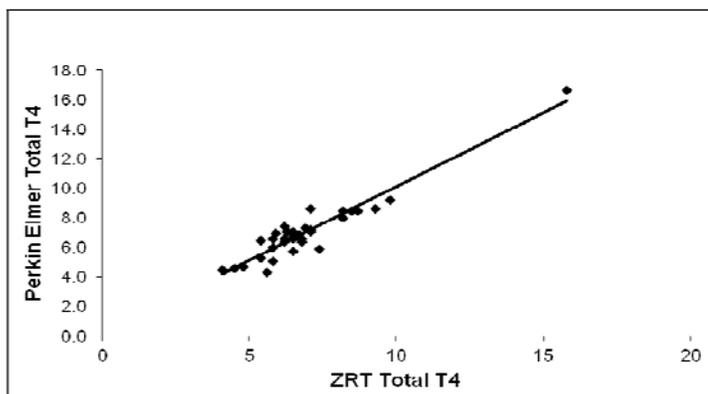
Inter-assay Precision

Inter-assay precision was determined by choosing three samples spanning the reference range, and analyzing them multiple times throughout different runs. Results are shown below:

Mean Total T4 Concentration (µg/dL)	Standard Deviation	Coefficient of Variation (C.V. %)
10.4	1.09	10.4
8.0	0.54	6.6
5.9	0.62	10.4

Accuracy

To test the accuracy of the dried blood spot assay for Total T4, dried blood spot samples collected at the same time were run by the ZRT method and by Perkin Elmer's Total T4 DELFIA kit procedure and analyzed by linear regression. Resulting correlation data are shown below ($R = 0.95$)



Analyte Stability

The dried blood spot samples are stable for more than 1 month at room temperature.

Specimen Collection

Kits for blood spot collection contain a filter paper collection card, finger lancets, an alcohol prep pad, sterile gauze, a band-aid, easy-to-follow instructions, and a mailer to return the sample for analysis.