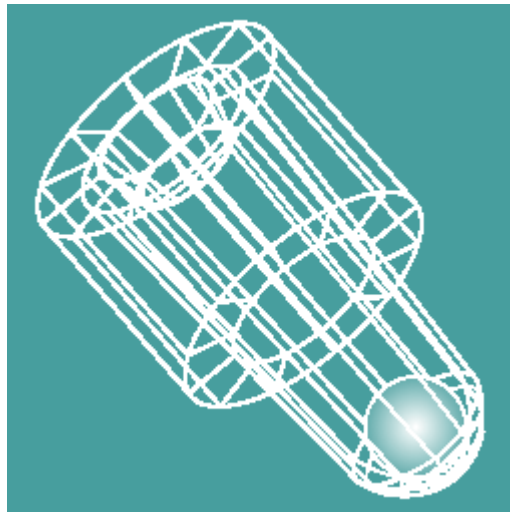


D P C Technical Report

I M M U L I T E[®]

Free T4 Assays: Analog and 2-Step



IMMULITE[®] Free T4 Assays: Analog and 2-Step

DPC now offers two tests to quantitatively measure non-protein-bound thyroxine (Free T4) levels in serum on the IMMULITE[®] chemiluminescent enzyme immunoassay system.

Explanation of the Test

The National Academy of Clinical Biochemistry has recommended the complementary use of Thyroid Stimulating Hormone (TSH) and Free Thyroxine (FT4) for the differential diagnosis and management of hypo- and hyperthyroidism¹. The principle thyroid hormone, thyroxine (T4), circulates almost entirely bound to carrier proteins² in an equilibrium which tends to reassert itself in the face of altered levels of carrier proteins by including a corresponding alteration in the total level of T4 in circulation, while leaving the unbound (free) T4 level relatively unchanged. Hence, free T4 concentration may be expected to correlate more closely to than the total T4 concentration with clinical thyroid status, as abnormal total T4 may signify either an abnormality in thyroid function, or simply a physiological or pathological variation in the carrier proteins. An example would be elevation in TBG levels in females during pregnancy, or when taking oral contraceptives or estrogen therapy causing total T4 levels to increase.

General IMMULITE[®] Procedure

The IMMULITE[®] assay works along the following basic principles. Patient serum and a ligand-labeled tracer are added to a test unit containing a polystyrene bead coated with an antibody specific to the analyte to be measured. After an incubation step, the test units undergo a centrifugal wash step, removing the residual sample and unbound analyte. An anti-ligand enzyme is then introduced, and the test unit undergoes an 2nd incubation, after which unbound enzyme is removed. A substrate is added, which, in the presence of the enzyme produces emission of photons, which are

measured by the IMMULITE[®] instrument, and converted into concentration.

Analog vs 2-Step

There are two major differences between the IMMULITE[®] Analog and 2-Step methods. The first is in the labeled components used to determine free T4 concentrations in each, and the second is the introduction of an intervening wash step in the 2 step assay.

The Free T4 Analog assay uses a ligand-labeled T4 analog that directly competes with endogenous Free T4 for binding sites on a polystyrene bead which has been coated with a T4 specific monoclonal antibody. The analog has been constructed so as to have no measurable affinity for thyroxine-binding globulin (TBG), the principal thyroid hormone transport protein. To prevent binding of the ligand-labeled analog to albumin, blocking agents are present, at a concentration carefully adjusted to avoid displacement of native T4 from endogenous carrier proteins. This method, however, is susceptible to the presence of rare interfering substances, such as anti-T4 antibodies. The presence of such interfering substances can potentially bind up the ligand-labeled analog, which could result in a smaller proportion of analog binding to the solid phase (the Ab-coated bead), thus artificially increasing the reported dose of endogenous FT4.

The 2-step assay avoids the effect of potential interfering substances by employing a ligand-labeled triiodothyronine (T3) molecule instead of a T4 analog, along with an introduction of an intermediate wash step. The Free T4 Analog assay introduces both the patient sample and the ligand-labeled analog into the IMMULITE[®] Test Unit simultaneously, resulting in a competition for binding sites on the solid-phase. In the 2-step assay, the patient sample is introduced first, allowing for non-competitive binding on the solid-phase. The test unit is then washed after 30 minutes,

removing all of the remaining sample including all unbound FT4 and any potentially interfering substance --including TBG, albumin and anti-T4 antibodies. Ligand-labeled T3 is then introduced and binds to the un-occupied sites on the solid-phase. The decision to use T3 instead of T4 for the labeled molecule was made because, although T3 will bind to a T4 antibody, it does not have as strong an affinity as T4, and thus no displacement of bound endogenous FT4 would take place.

Conclusion

Both the IMMULITE® Free T4 (Analog) and Free T4 (2-step) assays provide consistent and dependable FT4 results while adopting two distinct methodologies. DPC has provided our clients with this choice in test as a response to concerns and preferences voiced over specific needs seen in some clinical and research laboratories around the world. Although the two methodologies are distinct, intensive testing on the part of DPC has confirmed that the results obtained with each maintain a high degree of consistency across a very broad range of concentrations – from highly hypo- to hyperthyroid samples – and that regardless of which test is used, the client can be confident in the accuracy and dependability of the results.

¹ Sawin CT, ed. Standards of laboratory practice: Laboratory support for the diagnosis & monitoring of thyroid disease. National Academy of Clinical Biochemistry, 1996

² Wosilait WD. A theoretical analysis of the distribution of thyroxine among sites on thyroid binding globulin, thyroid binding prealbumin, and serum albumin. Res Commun Chem Pathol Pharmacol 1977; 16:541-8