QUALITY CONTROL

Each laboratory should assay controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant
deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

LIMITATIONS OF PROCEDURE

1. It is important that the time of reaction in each well is held constant for reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
4. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time-deviation during reaction.
5. Plate readers measure vertically. Do not touch the bottom of the wells.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.

INTERPRETATION

1. The T3-Uptake Test is dependent upon a multiplicity of factors: thyroid gland and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of the thyroid hormones to TBG. Thus, the T3-Uptake Test alone is not sufficient to assess clinical status.
2. The free thyroxine index (FTI), which is the product of the T-Uptake Ratio and the total thyroxine concentration, has gained wide clinical acceptance as a more accurate assessment of thyroid status. The FTI value compensates for any condition or drug, such as pregnancy or estrogens, which alters the TBG and the T4 levels but does not change the thyrometabolic status. A table of interfering drugs and conditions, which affect the T3-Uptake Test, has been compiled by the Journal of the American Association of Clinical Chemists.

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T3-Uptake (T3U) ELISA

Catalog No. IB19117 (96 tests)

INTENDED USE

The T3-Uptake ELISA used for the measurement of the total amount of binding sites available for the thyroid hormones in human serum or plasma. For research use only.

SUMMARY AND EXPLANATION

The thyroid gland under the regulatory control of thyrotropin hormone secretes thyroxine (T4) and triiodothyronine (T3) into the general circulation. The released hormones do not circulate as free molecules but are almost entirely (99.9%) bound to specific hormone binding serum proteins. Three protein fractions with varying affinities and capacities for interaction with T3 and T4 have been identified by reverse flow paper electrophoresis. Thyroxine binding globulin (TBG) carries 65~75% of the total circulating concentration. Thyroxine binding pre-albumin (TBPA) has an intermediate avidity for thyroxine (carries approx.15~25%) but little if any avidity for triiodothyronine. Albumin with a low affinity but high capacity carries 10% of thyroxine and 30% of the available triiodothyronine. Since the metabolic processes are regulated entirely by the concentration of the free thyroid hormones, which are inversely related to the levels of the binding proteins, an assessment of the binding capacity of human serum was developed in 1957 by Hamolsky (4). In this early method, radioactive T3 was added to a specimen of whole blood. After an incubation period, the mixture was centrifuged and the red cells washed. The radioactivity uptake of the red cells was inversely related to the binding capacity of the serum. Although this method had severe limitations, it proved to be a valuable diagnostic tool. Further technical improvements in the assay methodology of the T3-uptake test resulted as various separation agents such as coated charcoal (5), ion-exchange resins, denatured albumin, silicates, antibodies and organic polymers were employed in place of the red cells. This microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-T3 conjugate and thyroxine (T4) are added, and then the reactants are mixed. The endogenous binding proteins of the sample react with the thyroxine, but not with the enzyme conjugate. This leads to a higher binding of the enzyme conjugate to the antibody combining sites, reactive for triiodothyronine and thyroxine, immobilized on the well as the binding capacity of the specimen increases. After the completion of the required incubation period, the antibody bound enzyme-thyroxine conjugate is separated from the unbound enzyme-thyroxine conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color. The employment of several serum references of known unsaturated thyroid hormone binding capacity permits construction of a graph of absorbance and concentration. From comparison to the dose response curve, an unknown specimen's absorbance can be correlated with thyroid hormone binding capacity

PRINCIPLE OF THE TEST

The required components for assessing the binding capacity of human serum are enzyme-T3 conjugate, thyroxine, binding protein (P), and immobilized thyroxine antibody (Ab). Upon mixing the enzyme-conjugate and thyroxine with the specimen, a binding reaction results between the patient's binding proteins and the added thyroxine but not with the enzyme conjugate. The added thyroxine (T4) not consumed in reaction 1 then competes with the enzyme-T3 conjugate for a limited number of insolubulized binding sites. After equilibrium is attained, the antibody-bound fraction is separated from unbound enzyme-antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the binding capacity of the specimen. Thus, in hypothyroidism, the binding proteins are relatively unsaturated

(due to the low level of thyroid hormones) resulting in higher consumption of the added thyroxine than a euthyroid specimen. This leads to higher binding of the enzyme-triiodothyronine conjugate caused by the reduced concentration of the available thyroxine. In hyperthyroidism, the reverse is true. The binding proteins are relatively saturated with thyroxine (due to the high level of thyroid hormone) resulting in lower consumption of the added thyroxine. The remaining thyroxine is relatively much higher than an euthyroid specimen resulting in lower enzyme-antigen antibody binding due to the increased competition of the thyroxine for the limited antibody sites.

MATERIALS PROVIDED	96 Tests
1. Microwells coated with sheep anti-thyroxine serum	12x8x1
2. Calibrators – (4 vials)	1 ml
3. Enzyme-T3U Conjugate	1.5 ml
4. T3-Uptake Conjugate Buffer	13 ml
5. Wash Solution Concentrate	20 ml
6. Substrate A	7 ml
7. Substrate B	7 ml
8. Stop Solution (1 Vial)	8 ml

MATERIALS NOT PROVIDED

1. Pipette capable of delivering 25µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.300ml volumes with a precision of better than 1.5%.
3. Adjustable volume (20-200µl) and (200-1000µl) dispenser(s) for conjugate and substrate dilutions.
4. Microplate washers or a squeeze bottle (optional).
5. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
6. Absorbent Paper for blotting the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps. Timer. Quality control materials.

STORAGE AND STABILITY

1. Store the kit at 2 - 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
   The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. This test kit is designed for Research Use Only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that serum samples be run in duplicate.
6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

REAGENT PREPARATION:

1. Working Reagent A - T3U-enzyme Conjugate Solution

Dilute the T3U-enzyme conjugate 1:11 with T3 Uptake Conjugate Buffer in a suitable container. For example, dilute 160µl of conjugate with 1.6ml of buffer for 16 wells (A slight excess of solution is made). This reagent should be used within twenty-four hours for maximum performance of the assay.
Store at 2-8°C.

2. Wash Buffer

Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature 18 - 26°C for up to 60 days.

3. Working Substrate Solution

Pour the contents of the vial labeled Solution 'A' into the vial labeled Solution 'B'. Mix and store at 2-8°C. Use within 60 days. Or for longer periods of usage determine the amount of reagent needed and prepare by mixing equal portions of Substrate A and Substrate B in a suitable container. For example, add 1ml of A and 1ml of B per two (2) eight well strips (A slight excess of solution is made. Discard the unused portion). Note: Do not use the working substrate if it looks blue.

ASSAY PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18 - 26°C).

1. Format the microplate' wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipette 0.025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of Working Reagent A, T3U-enzyme solution to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 300µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells.
9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.