Monobind Inc.
Lake Forest, CA 92630, USA

VAST™
Versatile Analyte Selection Technology
3 Analytes 1 Kit
CLIA Microwells

Total Triiodothyronine, Total Thyroxine, Thyrotropin (T3/T4/TSH VAST®)

Thyroid Panel Test System

Product Code: 8075-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Total Thyroxine; Total Triiodothyronine; Thyroid Stimulating Hormone Concentration for a comprehensive thyroid status of a Human Serum or Plasma sample by a Microplate Enzyme Immunoassay, Chemiluminescence.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurements of thyroid hormones (tT3, tT4 and TSH) are generally regarded as invaluable *in-vitro* diagnostic tests for assessing thyroid function. This importance has provided the impetus for the significant improvement in assay methodology that has occurred in the last three decades. This procedural evolution can be traced from the empirical protein bound iodine (PBI) test[1] to the theoretically sophisticated radioimmunoassay[2] and currently used EIA, ELISA, FIA and Chemiluminescence.

The Combination Thyroid Panel (CTP) provides the convenience of combination calibrators, universal plate and flexible reagent selection allowing technicians to perform a variety of assay designs. In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-tT4 (tT3) conjugate and biotinylated tT4 or tT3 antibody are added, and the reactants are mixed. In the case of TSH, the biotinylated and enzyme conjugate are added in one step. A reaction results between the enzyme conjugate, biotinylated conjugate and the native thyroid hormone (tT3, tT4 or TSH) for the antibody combining sites. Immobilization takes place through the reaction of the incorporated biotin and streptavidin coated on the well. After the completion of the required incubation period, the bound enzyme conjugate is separated from the unbound enzyme 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 light.

The employment of several serum references of known thyroid hormone concentration(s) permits construction of a graph of activity and concentration. From comparison to the dose response curve(s), an unknown specimen's activity can be correlated with hormone concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (tT3 and tT4) – Type 7
The essential reagents required for a chemiluminescence immunoassay include antibody, enzyme-antigen conjugate, native antigen and a substrate that emits light.

Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the followed equation:

$$^{Enz}Ag + Ag + Ab_{Btn} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{Btn} + {}^{Enz}AgAb_{Btn}$$

$Ab_{C.W}$ = Monospecific Immobilized Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{Btn}$ = Antigen-Antibody Complex
$^{Enz}AgAb_{Btn}$ = Enzyme-antigen Conjugate –Antibody Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.
$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin}_{CW} \Rightarrow$ <u>immobilized complex</u>
$\underline{Streptavidin}_{CW}$ = Streptavidin immobilized on well
<u>Immobilized complex</u> = sandwich complex bound to the solid surface

The enzyme activity in the antibody-bound fraction, measured by reaction with luminol, is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

Immunoenzymometric assay (TSH) - TYPE 3
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-TSH antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, a reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(p)} + Ag_{TSH} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(p)} - Ag_{TSH} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
Ag_{TSH} = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(p)}$ =Enzyme -Polyclonal Antibody (Excess Quantity)
$^{Enz}Ab_{(p)} - Ag_{TSH} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab_{(p)} - Ag_{TSH} - {}^{Btn}Ab_{(m)} + \underline{Streptavidin}_{CW} \Rightarrow$ <u>immobilized complex</u>
$\underline{Streptavidin}_{CW}$ = Streptavidin immobilized on well
<u>Immobilized complex</u> = sandwich complex bound to the solid surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction, measured by reaction with luminol, is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Material provided:

A. Combi-Cal® T3/T4/TSH Calibrators - 1ml/vial - Icons A-F
Six (6) vials of Thyroid Combi-Cal® human serum calibrators dispensed in vials with the concentrations as listed in the Table. Store at 2-8°C. A preservative has been added

Analyte	Total T3 (ng/ml)	Total T4 (µg/dl)	TSH (µIU/ml)
A	0	0	0
B	0.5	2.0	0.5
C	1.0	5.0	2.5
D	2.5	10.0	10.0
E	5.0	15.0	20.0
F	7.5	25.0	40.0

TSH concentrations were calibrated using a reference preparation, which was assayed against the WHO 2nd. IRP 80/558

B. T4 Tracer Reagent – 1ml/vial - Icon Ⓔ
One (1) vial containing thyroxine-horseradish peroxidase (HRP) conjugate in a serum albumin-stabilizing matrix. A preservative has been added. Store at 2-8°C.

C. T3 Tracer Reagent – 1ml/vial - Icon Ⓔ
One (1) vial containing triiodothyronine -horseradish peroxidase (HRP) conjugate in a serum albumin-stabilizing matrix. A preservative has been added. Store at 2-8°C

D. s-T3/T4 Buffer - 13ml/vial - Icon SCR
One (1) vial reagent containing buffer, red dye, preservative, and binding protein inhibitors. Store at 2-8°C.

E. TSH Tracer Reagent - 20ml/vial - Icon Ⓔ
One (1) vial containing enzyme labeled affinity purified polyclonal goat antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

F. T4 Biotin Reagent – 7ml/vial – Icon ∇
One (1) vial containing biotinylated anti-thyroxine (sheep) reagent in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

G. T3 Biotin Reagent – 7ml/vial – Icon ∇
One (1) vial containing biotinylated anti-triiothyronine (sheep) reagent in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

H. Light Reaction Wells – 2 x 96 wells – Icon ⇓
Two 96-well microplates coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

I. Wash Solution Concentrate -- 20ml/vial - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

J. Signal Reagent A – 2 x 7ml/vial - Icon C^A
Two (2) vials containing luminol in buffer. Store at 2-8°C. See "Reagent Preparation."

K. Signal Reagent B - 2 x 7ml/vial - Icon C^B
Two (2) vials containing hydrogen peroxide in buffer. Store at 2-8°C. See "Reagent Preparation."

L. Product Insert

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**

Note 3: Above reagents are for a 192-well microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.025 & 0.050ml (25 & 50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Adjustable volume (20-200µl) and (200-1000µl) dispenser(s) for conjugate dilutions.
4. Microplate washer or a squeeze bottle (optional).
5. Microplate Luminometer
6. Test tubes for dilution of tracer conjugate and Signals A and B.
7. Absorbent Paper for blotting the microplate wells.
8. Plastic wrap or microplate cover for incubation steps.
9. Vacuum aspirator (optional) for wash steps.
10. Timer.
11. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum or plasma 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 (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8ºC for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20ºC for up to 30 days. Avoid use of contaminate devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required for tT4 and 0.100ml (100µl) for tT3 and TSH.

7.0 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. The individual laboratory should set acceptable assay performance limits. In addition, maximum RLU should be consistent with past experience. 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.

8.0 REAGENT PREPARATION

1. **Working Reagent A = tT4 or (tT3) - Tracer Solution**
   Dilute the T4-Tracer (or T3 Tracer) 1:11 with s-T3/T4 buffer in a suitable container. For example, dilute 0.080ml (80µl) of conjugate with 0.800ml (800µl) 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.
   General Formula:
   Amount of s-T3/T4 Buffer required = Number of wells ∗ 0.05
   Quantity of tT4 Tracer Reagent necessary = # of wells ∗ 0.005
   i.e. = 16 x 0.05 = 0.8ml (80µl) for s-T3/T4 Buffer
   16 x 0.005 = 0.08ml (80µl) for tT4 or (tT3) Tracer Reagent
2. **Wash Buffer**
   Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C.
3. **Working Signal Reagent Solution -** Store at 2 - 8°C.
   Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1ml of B per two (2) eight well strips (A slight excess of solution is made). **Discard the unused portion if not used within 36 hours after mixing.** If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

Note: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20 - 27°C).
*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum calibrator, 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 0.025ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well for tT4**. Pipette 0.050ml (50µl) for tT3. Pipette 0.050ml (50µl) for TSH.**
3. Add 0.050 ml (50µl) of Working Reagent A, tT4 **or tT3** Tracer solution to the appropriate wells (see Reagent Preparation Section). **For TSH, add 0.100 of TSH Tracer Reagent and skip steps 4 and 5.**
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Add 0.050 ml (50µl) of x-T4 **or (x-T3)** Biotin Reagent solution to the appropriate wells.
6. Swirl the microplate gently for 20-30 seconds to mix and cover.
7. Incubate 45 minutes at room temperature.
8. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
9. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) 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 four (4) additional times.**
10. Add 0.100ml (100µl) of working signal reagent solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE PLATE AFTER SIGNAL ADDITION

11. Incubate for five (5) minutes at room temperature in the dark.
12. Read the Relative Light Units (RLUs) in each well in a microplate luminometer for at least 0.2 seconds/well. **The results can be read within 30 minutes of adding the signal solution.**

Note: For reassaying specimens with concentrations greater than highest calibrator, dilute 0.0125ml (12.5µl - tT4) or 0.025ml (25µl - tT3-TSH) of the specimen and 0.0125ml (12.5µl - tT4) or 0.025ml (25µl -tT3-TSH) of the 0 serum reference into the sample well (this maintains a uniform protein concentration). Multiply the readout value by 2 to obtain the thyroxine concentration.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of thyroid hormones in unknown specimens.

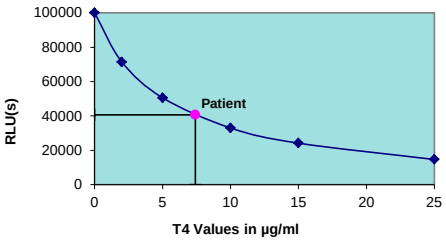
1. Record the RLUs obtained from the printout of the luminometer as outlined in Example 1 – tT4, Example 2 - tT3 or Example 3 - TSH.
2. Plot the RLUs for each duplicate serum reference versus the corresponding thyroid hormone concentration in the appropriate units on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve (Figures 1-3).
4. To determine the concentration of tT4 (tT3 – TSH) for an unknown, locate the average RLUs of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration in µg/dl tT4 (ng/ml tT3 - µIU/ml TSH) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLU 40596 intersects the calibrator curve at 7.4 µg/dl tT4 concentration (See Figure 1).

Note: Computer data reduction software designed for chemiluminescence assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1 – tT4

Sample I.D.	Well Number	RLUs	Mean RLUs	Value (ng/ml)
Cal A	A1	100645	100000	0
	B1	99355		
Cal B	C1	72597	71454	2.0
	D1	70311		
Cal C	E1	50446	50567	5.0
	F1	50692		
Cal D	G1	33219	32995	10.0
	H1	32771		
Cal E	A2	23993	24152	15.0
	B2	24313		
Cal F	C2	14616	14650	25.0
	D2	14684		
Ctrl 1	E2	47395	47876	**5.5**
	F2	48357		
Ctrl 2	G2	25583	25228	**14.3**
	H2	24872		
Patient	A3	41113	40596	**7.4**
	B3	40080		

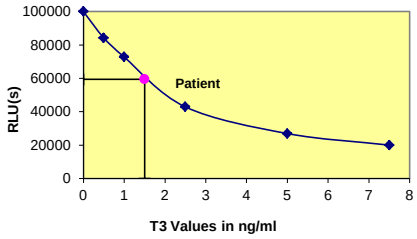
Figure 1



EXAMPLE 2 – tT3

Sample I.D.	Well Number	RLU	Mean RLUs	Value (ng/ml)
Cal A	A1	98034	100000	0
	B1	101966		
Cal B	C1	84125	84211	0.5
	D1	84211		
Cal C	E1	73866	72767	1.0
	F1	71668		
Cal D	G1	42894	42894	2.5
	H1	43286		
Cal E	A2	26861	26930	5.0
	B2	26999		
Cal F	C2	19622	20000	7.5
	D2	20368		
Ctrl 1	E2	74933	74929	**0.9**
	F2	74924		
Ctrl 2	G2	47321	46620	**2.2**
	H2	45920		
Patient	A3	58859	59352	**1.5**
	B3	59846		

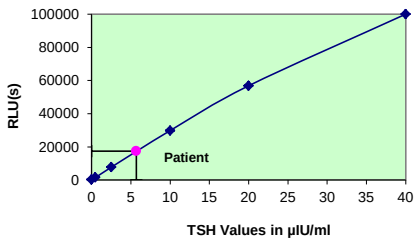
Figure 2



EXAMPLE 3 - TSH

Sample I.D.	Well Number	RLU	Mean RLUs	Value (µIU/ml)
Cal A	A1	248	269	0
	B1	291		
Cal B	C1	1562	1551	0.5
	D1	1539		
Cal C	E1	7823	7750	2.5
	F1	7677		
Cal D	G1	29528	29820	10
	H1	30112		
Cal E	A2	56758	56851	20
	B2	56944		
Cal F	C2	100241	100000	40
	D2	99759		
Patient	A3	17355	17488	**5.7**
	B3	17622		

Figure 3



The data presented in Example1-3 and Figure 1-3 are for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay. In addition, the RLUs of the calibrators have been normalized to 100,000 RLUs for the F calibrator for TSH and A calibrator for T3 and T4 (greatest light output). This conversion minimizes differences caused by efficiency of the various instruments that can be used to measure light output.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The dose response curve should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal reagent initiates a kinetic reaction; therefore, the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
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.
8. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
9. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
10. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
11. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (Boscato LM Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin.Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. Total serum thyroxine concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of thyroxine to TBG.[3,4] Thus, total thyroxine concentration alone is not sufficient to assess clinical status.
8. Total serum thyroxine values may be elevated under conditions such as pregnancy or administration of oral contraceptives. A fT3 uptake test may be performed to estimate the relative TBG concentration in order to determine if the elevated fT4 is caused by TBG variation.
9. A decrease in total thyroxine values is found with protein-wasting diseases, certain liver diseases and administration of testosterone, diphenylhydantoin or salicylates. A table of interfering drugs and conditions, which affect total thyroxine values, has been compiled by the Journal of the American Association of Clinical Chemists.
10. Total serum thyroxine values may be elevated under conditions such as pregnancy or administration of oral contraceptives. A tT3 uptake test may be performed to estimate the relative TBG concentration in order to determine if the elevated tT4 is caused by TBG variation.
11. A decrease in total thyroxine values is found with protein-wasting diseases, certain liver diseases and administration of testosterone, diphenylhydantoin or salicylates. A table of interfering drugs and conditions, which affect total thyroxine values, has been compiled by the Journal of the American Association of Clinical Chemists.

"NOT INTENDED FOR NEWBORN SCREENING"

13.0 EXPECTED RANGES OF VALUES

A study of euthyroid adult population was undertaken to determine expected values. The mean (R) values, standard deviations (**σ**) and expected ranges (±2**σ**) are presented in Table 1 for tT3 and Table 2 for tT4. A nonparametric method (95% Percentile Estimate) was used for TSH in Table 3.

TABLE 1 - Expected Values – (tT3) - In ng/ml	
Mean (X)	**1.250**
Standard Deviation (σ)	0.375
Expected Ranges (±2σ)	0.50 – 2.00
Number	105

TABLE 2 - Expected Values – (tT4) - In µg/dl		
	Male	**Female ***
Mean (X)	7.6	8.2
Std.Dev (σ)	1.6	1.7
Expected Ranges (±2σ)	4.4 – 10.8	4.8 – 11.6
Number	42	58

*Normal patients with high TBG levels were **not** excluded except if pregnant.

TABLE 3 - Expected Values – (TSH) - in µIU/ml	
Number	**139**
Low Normal Range	0.39
High Normal Range	6.16
70% Confidence Intervals for 2.5 Percentile	
Low Range	**0.28 – 0.53**
High Range	5.60 – 6.82

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: specificity of the method, population tested and precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the Total T3/Total T4/TSH VAST® CLIA test systems were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 4 and Table 5. The precisions for TSH VAST® CLIA are displayed in Table 6 and 7.

TABLE 4
Within Assay Precision for tT4 (µg/dl) and **tT3 (ng/ml)**

Sample	N	X	σ	C.V.
Low	16	2.9 **(0.81)**	0.17 **(0.05)**	5.8% **(7.29%)**
Normal	16	9.1 **(1.86)**	0.22 **(0.08)**	2.4% **(4.3%)**
High	16	15.6 **(3.15)**	0.65 **(0.12)**	4.2% **(3.6%)**

TABLE 5
Between Assay Precision for tT4 (µg/dl) and **tT3 (ng/ml)**

Sample	N	X	σ	C.V.
Low	10	2.8 **(0.78)**	0.23 **(0.07)**	8.2% **(9.0%)**
Normal	10	8.8 **(1.91)**	0.52 **(0.16)**	5.9% **(8.4%)**
High	10	15.9 **(3.23)**	0.78 **(0.23)**	4.9% **(7.1%)**

*As measured in ten experiments in duplicate over a ten day period.

TABLE 6
Within Assay Precision for TSH (µIU/ml)

Sample	N	X	σ	C.V.
Pool 1	24	0.42	0.03	7.1%
Pool 2	24	6.15	0.39	6.3%
Pool 3	24	27.80	1.76	6.3%

TABLE 7
Between Assay Precision for TSH (µIU/ml)

Sample	N	X	σ	C.V.
Pool 1	10	0.45	0.04	8.9%
Pool 2	10	6.03	0.48	8.0%
Pool 3	10	28.20	1.37	4.9%

*As measured in ten experiments in duplicate over seven days.

14.2 Sensitivity

The tT3 procedure has a sensitivity of 0.02 ng/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

The tT4 procedure has a sensitivity of 50 pg. This is equivalent to a sample containing a concentration of 0.2 µg/dl. The sensitivity was ascertained by determining the variability of the 0 µg/dl serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

The TSH procedure has a sensitivity of 0.05 µIU//ml. The sensitivity (detection limit) was ascertained by determining the variability of the 0 µIU/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The Total T4 / Total T3 / TSH VAST® AccuLite® CLIA Test System was compared with reference methods. The least square regression equation and the correlation coefficient were computed for the CLIA in comparison with the reference methods. The data obtained are displayed in Table 8-10.

TABLE 8 (tT3)

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method	1.45	y = 2.5 + 0.965(x)	0.959
Reference	1.52		
Number: 90		**Range of values**	0.15 – 8.0

TABLE 9 (tT4)

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	7.82	y = 0.28+0.964(x)	0.967
Reference	7.65		
Number: 85		**Range of values**	0.8 – 25

TABLE 10 (TSH)

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method	4.32	y = 0.34 + 0.981 (x)	0.989
Reference	4.49		
Number: 110		**Range of values**	0.01 – 61

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross-reactivity of the antibodies used to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of thyroid hormone needed to displace the same amount of tracer.

tT3

Substance	Cross Reactivity	Concentration
l-Triiodothyronine	1.0000	-
l-Thyroxine	< 0.0002	10µg/ml
Iodothyrosine	< 0.0001	10µg/ml
Diiodothyrosine	< 0.0001	10µg/ml
Diiodothyronine	< 0.0001	10µg/ml
Phenylbutazone	< 0.0001	10µg/ml
Sodium Salicylate	< 0.0001	10µg/ml

tT4

Substance	Cross Reactivity	Concentration
l–Thyroxine	1.0000	-
d-Thyroxine	0.9800	10µg/dl
d-Triiodothyronine	0.0150	100µg/dl
l–Triiodothyronine	0.0300	100µg/dl
Iodothyrosine	0.0001	100µg/ml
Diiodothyrosine	0.0001	100µg/ml
Diiodothyronine	0.0001	100µg/ml

TSH

Substance	Cross Reactivity	Concentration
Thyrotropin (hTSH)	1.0000	-
Follitropin (hFSH)	< 0.0001	1000ng/ml
Lutropin Hormone (hLH)	< 0.0001	1000ng/ml
Chorionic Gonadotropin (hCG)	< 0.0001	1000ng/ml

15.0 REFERENCES

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MP8075 Product Code: 8075-300

Size		192(B)	480(D)	960(E)
Reagent (fill)	A)	1ml set	2.75ml set	2(2.75ml set)
	B)	1(1ml)	1(2ml)	2(2ml)
	C)	1(1ml)	1(2ml)	2(2ml)
	D)	1(13ml)	1(30ml)	1(30ml)
	E)	1(20ml)	1(35ml)	1(60ml)
	F)	1(7ml)	1(15ml)	2(15ml)
	G)	1(7ml)	1(15ml)	2(15ml)
	H)	2 plates	5 plates	10 plates
	I)	1(20ml)	1(60ml)	1(60ml)
	J)	2(7ml)	1(55ml)	2(55ml)
	K)	2(7ml)	1(55ml)	2(55ml)

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