

51 0(k) Summary of Information Respecting Safety and Effectiveness

K-952751

A. **Submitter:**

Biocircuits Corporation
1324 Chesapeake Terrace
Sunnyvale, CA 94089
(408) 745-1961
Contact: Sheila Ramerman
Date Prepared: October 4, 1995

10/20/1995

B. **Device Names:**

Proprietary Name: Biocircuits IOS™ T4/TU Test Cartridges
Biocircuits IOS™ Thyroid Controls

Common Names: Reagents for total thyroxine assay
Reagents for thyroid uptake assay
Quality control material (assayed and unassayed)

Classification Name: Total thyroxine test system
Triiodothyronine uptake test system
Quality control material (assayed and unassayed)

c. **Legally Marketed Device:**

The IOS™ T4/TU Test Cartridges are substantially equivalent to the Stratus T4 and TU tests currently in commercial distribution by Dade International.

D. **Device Description:**

Thyroxine (T4) is a hormone that is synthesized and stored in the thyroid gland, plays a vital role in growth and maturation, and is essential in the control of metabolism in all bodily organs. More than 99% of T4 is reversibly bound to three plasma proteins in the blood: **thyroxine-binding globulin (TBG)**, **thyroxine-binding prealbumin (TBPA)**, and albumin. The balance of T4 is in the free, unbound state in blood at any one time. (1)

Malfunction of the thyroid gland may result in abnormal concentrations of T4. Increased levels of T4 have been found in hyperthyroidism due to Grave's disease, toxic multinodular goiter, and in acute and subacute thyroiditis. Low levels of T4 have been associated with congenital hypothyroidism, myxedema, chronic thyroiditis (Hashimoto's disease), and with some genetic abnormalities. (2)

Measurement of T4 concentration alone fails to take into account any variation in TBG levels which can affect the free T4 concentration. The Thyroid Uptake (T-Uptake) test was developed to produce an indirect measure of the unsaturated TBG in the specimen. The product of T4 and T-Uptake is the Free Thyroxine Index (FTI). The FTI correlates more closely with free T4 levels, and is a better method of monitoring thyroid function and diagnosing thyroid illness than a T4 determination alone. (3,4,5)

Principle of the Test:

T4: The IOS™ T4 test is a competitive immunoassay in which T4 in the sample is first released from the carrier proteins by 8-anilino-1-naphthalene sulphonic acid (ANS) and salicylate, and then competes with alkaline phosphatase-labeled T4 (conjugate) for binding sites on immobilized monoclonal anti-T4 antibody. After a short incubation, excess sample and conjugate are washed away. Substrate is added, which reacts with the alkaline phosphatase conjugate bound to antibody. The product of the enzyme-substrate reaction produces a fluorescent signal. The level

of fluorescence is directly proportional to the amount of conjugate bound to the surface, and inversely proportional to the amount of T4 present in the patient sample. All reagents necessary to perform the test are dried in the IOS™ cartridge, and are dehydrated by the addition of patient sample by the operator, or by the addition of buffer by the instrument.

T-Uptake: The IOS™ T-Uptake test is a competitive immunoassay in which patient sample is mixed with exogenous T4, which binds to TBG in the patient sample. When serum containing unsaturated TBG is present in the system, some of the exogenous T4 will bind to TBG; conversely, when serum containing more fully saturated TBG is present, the amount of exogenous T4 in solution is increased. Patient sample containing the exogenous T4 is also mixed with alkaline phosphatase-labeled T4 (conjugate); this mixture is then incubated with immobilized monoclonal anti-T4 antibody. Any exogenous T4 not bound to patient TBG and conjugate compete for the binding sites on the antibody. After a short incubation, excess mixture is washed away. Substrate is added, which reacts with the alkaline phosphatase conjugate that is bound to antibody. The product of the enzyme-substrate reaction produces a fluorescent signal. The amount of signal produced is directly proportional to the amount of conjugate bound to the antibody; the amount of conjugate bound is inversely proportional to the amount of exogenous T4 available to bind to the antibody, or the amount of exogenous T4 'uptake' by the antibody. This amount of exogenous T4 is inversely proportional to the amount of exogenous T4 which was bound to the patient TBG. For example, more saturated TBG present in the patient sample to begin with means less exogenous T4 to be bound by the patient sample. Less exogenous T4 bound to patient TBG means more is available to be 'taken up' by antibody binding sites and less conjugate to bind to the antibody, thus producing a low signal and a high 'uptake' value. A low uptake value means the reverse is true: the patient sample contained less saturated TBG to begin with.

Thyroid Controls: The use of materials derived from human blood to monitor quality control of clinical chemistry testing in the clinical laboratory has been widely established over the past several years. (1) The Biocircuits IOS™ Thyroid Controls are two levels of blood-based material for use with Biocircuits IOS™ thyroid assays test cartridges. Controls are provided to monitor the hypothyroid and hyperthyroid clinical decision levels in the IOS™ thyroid assays.

To run a control, the operator inserts the Thyroid Control Cartridge (packaged with the controls) into the IOS™ instrument. The instrument reads the lot number and ranges of acceptable values for the control solutions from the Control Cartridge barcode, and then ejects the Control Cartridge. The operator then inserts a T4/TU test cartridge and follows the instrument prompts to identify the control level, apply control solutions, and begin the test sequence. The IOS™ instrument performs the required buffer additions to rehydrate assay reagents and perform wash steps as necessary, reads the fluorescence signal generated, and calculates and prints the control result just as it would if the cartridge were used to test a patient sample.

E. Intended Use

The Biocircuits IOS™ T4/T-Uptake (T4/TU) cartridge is to be used for the quantitative determination of total thyroxine levels and the degree of saturation of thyroid binding proteins in serum in the Biocircuits IOS™ System.

The IOS™ Thyroid Controls Kit is to be used to assist in monitoring accuracy and precision in the IOS™ thyroid assays.

F. Comparison with the Predicate Device:

Table I summarizes the comparative features of both the IOS™ and Stratus T4 and TU assays.

G. Performance Data:

1. T4/TU Test Cartridges:

Non-clinical testing performed in the manufacturer's laboratories gave the following results:

Sensitivity: The analytical sensitivity of the IOS™ T4 assay is defined as the smallest concentration of T4 that can be distinguished from zero. Ten replicates of a zero calibrator were run using IOS™ T4/TU cartridges (only the result from the T4 track was recorded). The mean value and standard deviation of the signal were calculated. The sensitivity was obtained by interpolating two standard deviations above the mean signal of the zero calibrator. The sensitivity of the IOS™ T4 assay is 1.85 ug/dL.

Specificity: Cross-Reactivity

The specificity of the monoclonal antibody used in both the IOS™ T4 and T-Uptake assays was determined by evaluating the cross-reactivity with substances similar in structure to T4 or those known to interfere with the immunological reaction. Results are listed below for the T4 assay; because TU provides a relative assessment of the unsaturated binding capacity of serum proteins for thyroid hormones, there are no known cross-reactivities for a TU test. Pooled human serum with a value of 8.5 ug/dL T4 was used.

Compound	Cross-Reactivity
D-thyroxine (D-T4)	100%
L-triiodothyronine (L-T3)	< 49(0)
D-triiodothyronine (D-T3)	< 270
3,5-diiodothyronine	< 0.190
3,5-diiodotyrosine	< 0.1%
Phenytoin (diphenylhydantoin), at 10,000 ug/dL	< 0.170
Propylthiouracil, at 10,000 ug/dL	< 0.170
Phenylbutazone, at 10,000 ug/dL	< 0.190
Salicylic acid, at 50 mg/dL	< 0.170
Acetylsalicylic acid (AS A), at 50 mg/dL	< 0.170

Specificity: Interfering Substances

The following substances were tested at the levels specified and found not to significantly affect the T4 or TU result obtained using the IOS™ T4/TU cartridge. A pool of human serum with values of 5.8 ug/dL T4 and 30.8% TU was used.

Substance	Interference: T4	TU
Hemoglobin @ 500 mg/dL	6%	-2%
Bilirubin (unconjugated) @ 20 mg/dL	6%	9%
cholesterol @ 500 mg/dL	1%	-3%
Triglycerides @ 750 mg/dL	4%	-4%

Precision: The following results were obtained from a laboratory study performed at the manufacturer for within-day, between-day, and total imprecision:

T4			
Control Level	1	2	3
Mean (ug/dL)	8.1	12.9	4.65
SD, overall (ug/dL)	0.72	1.14	0.56
% CV, within-day (n=10)	8.5%	8.8%	14.4%
% CV, between-day (n=40)	5.0%	4.4%	6.9%
% CV total	8.9%	8.8%	12.1%
T-Uptake			
Control Level	1	2	3
Mean (% uptake)	32.4	39.7	22.82
SD, overall (% uptake)	1.04	0.93	1.78
% CV within-day (n=10)	2.9%	2.4%	3.8%
% CV between-day (n=40)	2.0%	1.1%	5.9%
% CV total	3.2%	2.3%	7.8%

Accuracy: Spiked Recovery

A normal human serum pool with a known T4 value was spiked with different quantities of T4 and then assayed in duplicate using IOS™ T4/TU cartridges (only the T4 track was used). The results are as shown:

Endogenous [T4] (ug/dL)	Added [T4] (ug/dL)	Average Observed (ug/dL)	Average Recovery(%)
4.25	3.5	7.55	93%
4.25	6.0	10.2	99%
4.25	9.2	13.5	100%
4.25	12.4	16.5	99%
4.25	18.0	20.7	91%

Accuracy: Cm-relation

A comparison of methods obtained by testing 126 patient samples in the manufacturer's enzyme laboratories UK using the IOS™ T4/TU cartridges and a commercially available immunoassay gave **the following results, in the form $y = b + mx$, with "r" being the correlation coefficient.**

The samples tested ranged from 1.8 ug/dL to 24.6 ug/dL T4.

T4: $y = 0.229 + 0.943x, r = 0.937$
TU: $y = 7.120 + 0.748x, r = 0.703$
FTI: $y = -0.037 + 0.959x, r = 0.932$

Clinical testing performed at a typical physicians' office laboratory gave the following results:

Precision

T4 Control Level	1	2	3
number of replicates			
Mean (ug/dL)	7.46	11.75	4.79
SD, overall (ug/dL)	0.5	0	1.01
% CV, total	10.1%	11.9%	21.2%

T-Uptake

control Level	1	2	3
number of replicates	9	4	
Mean (% uptake)	32.24	39.63	24.54
SD, overall (% uptake)	1.46	1.06	2.93
% CV, total	4.5%	2.7%	12.0%

Accuracy: Correlation

A comparison of methods was performed by users in a typical physicians' office laboratory. A total of 43 patient samples were tested using the IOS™ T4/TU cartridges in the office laboratory; the samples were split and sent to the manufacturer's laboratory for retesting on both the IOS™ and on the predicate device. These studies gave **the following results, in the form $y = b + mx$, with "r" being the correlation coefficient.**

The samples tested ranged from 1.8 ug/dL to 20.6 ug/dL T4.

T4: $y = 1.915 + 0.771x, r = 0.919$
TU: $y = 3.470 + 0.878x, r = 0.821$
FTI: $y = 1.814 + 0.775x, r = 0.927$

Expected Values:

In a reference range study of 125 normal, healthy adults (98 males and 27 females) in the manufacturer's laboratories, the range of T4 values for the IOS™ T4 assay was determined to be 4.6-103 ug/dL.

25

In a reference range study of 125 normal, healthy adults (98 males and 27 females) in the manufacturer's laboratories, the range of T-Uptake values for the IOS™ T-Uptake assay was determined to be 25.6 -32.9 % .

In a reference range study of 125 normal, healthy adults (98 males and 27 females) in the manufacturer's laboratories, the range of FTI values for the IOS™ T4/TU assays was determined to be 3.9- 11.3

T4 and FTI levels are generally depressed in hypothyroid patients and generally elevated in hyperthyroid patients. T4 levels may also be elevated in neonates and infants.

Since T4 and T⁴-Uptake values (and, therefore, FTI values) may vary with many factors, such as geographic location, local population, diet, etc., these ranges are provided for general informational purposes and may not apply to your specific patient population. We recommend that each laboratory establish its own reference ranges for its patient population.

2. Thyroid Controls

The following ranges for the IOS™ Thyroid Controls were determined in studies in the manufacturer's laboratories. To establish the ranges, the controls were tested in a total of 40 cartridges each, over at least 10 days, using three IOS™ instruments. These values only apply to this lot of IOS™ Thyroid Controls. Different lots of Thyroid Controls will likely have slightly different ranges. Your laboratory should establish its own range for these controls over time.

Control	T4 (ug/dL)		T-Uptake (%)	
	Mean	Range (± 2 SD)	Mean	Range (± 2 SD)
1	8.1	6.7- 9.5	32.4	30.3 -34.5
2	12.9	10.7 -15.2	39.7	37.8 -41.6

It is self-evident from the data and information presented here that the Biocircuits IOS™ T4/TU Test Cartridges are as safe, as effective, and perform as well as the Stratus T4 and TU assays in commercial distribution by Dade International.

Attachment: Table 1: Assay Comparison

TAB 1
 Baxter STRATUS vs. Biocircuits IOS™
 Assay Comparison

<u>ATTRIBUTE</u>	<u>STRATUS T4/TU</u>	<u>IOS T4/TU</u>
Technology	Fluorometric enzyme immunoassay	Fluorometric enzyme immunoassay
Assay format	Sequential/sequential	Competitive/competitive
Enzyme label	Alkaline phosphatase	Alkaline phosphatase
Substrate	Methylumbelliferyl phosphate	Methylumbelliferyl phosphate
Reagents		
In-mobilization Medium	Reaction tab	Plastic cartridge
Dry	Monoclonal antibody only	Monoclonal antibody, ANS/salicylate, T4-AP, substrate
Wet	3, loaded by operator at start of each run	1 (same for all assays), continuously on board
Delivery	Fully automated	Fully automated
Calibration	User-generated	Factory-generated
Calibration Stability	14 days (minimum)	30 days (minimum)
Storage	Refrigerated (2-8°C)	Room Temperature (15-30°C)
Sample		
Type	Serum or plasma	Serum
Volume	0.2 ml (minimum)	0.033 mL
Dilution	Performed by instrument	Performed by instrument
Operating environment	22°-320 C	15°-300 c
Data analysis	Microprocessor-controlled Stored standard curves	Microprocessor-controlled Stored standard curves
Data output	LCD display Printed alphanumeric hard copy	LCD display Printed alphanumeric hard copy