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E. R. Squibb & Sons, Inc.

P.O. Box 191
New Brunswick, New Jersey 08903
201-545-1300

CHARLES L. KROLL, Sc. D., director
regulatory operations



SQUIBB

December 18, 1980



Radioisotope Licensing Branch
Division of Fuel Cycle and Material Safety
U. S. Nuclear Regulatory Commission
Washington, D. C. 20555

Gentlemen:

Reference is made to our specific license 29-00139-05G, pursuant to 10 CFR 32.71, to manufacture or distribute by-product material for use under the general license of 10 CFR 31.11.

We now wish to request renewal of this license which expires on January 31, 1981, for the following products, all of which contain Iodine 125 not exceeding 10 microcuries in each prepackaged unit:

1. Angiotensin I IMMUTOPE Kit, 200 test kits, List 09421
2. Digoxin CLASP RIA Kit, 100 test kit, List G1330
3. ESTRIOL-SQUIBB RADIOIMMUNOASSAY Kit, 100 test kit, List H0810
4. HTSH CLASP RIA Kit, 100 test kit, List G2120
5. THYROSTAT-3 Diagnostic Test Kit, 25 test kit, List 09026
6. THYROSTAT-3 Diagnostic Test Kit, 100 test kit, List 09028
7. T3 CLASP RIA Kit, 100 test kit, List G2105
8. T3-SQUIBB RADIOIMMUNOASSAY Kit, 100 test kit, List H0820
9. T4 CLASP RIA Kit, 100 test kit, List G2110
10. THYROSTAT-FTI Diagnostic Test Kit, 25 test kit, List 09127
11. THYROSTAT-FTI Diagnostic Test Kit, 100 test kit, List 09152
12. THYROSTAT FTI Diagnostic Test Kit, 5 x 100 test kit, List 09172



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The printed labeling components which contain the information required under §32.71(c)(1) and (2), §32.71(d), and §32.71(e) are enclosed for each product. For the purposes of this application, only those portions of the brochures, which accompany the packages, containing information pertinent to §32.71(d) and (e) are being submitted for review; these are highlighted in the attached brochures.

In addition, a check for $570.00 is enclosed to cover the required renewal fee.

We trust this information is adequate to support renewal of our license 29-00139-05G.

Sincerely,

C Kroll

C. L. Kroll

CLK:js
Enclosure

06242


200 ml List 09421

# $^{125}$I ANGIOTENSIN I BUFFER

Approx. 0.005 μg Angiotensin I per 200 ml

For *in vitro* Diagnostic Use • See insert

Not for Internal or External Use in Humans or Animals

**SODIUM AZIDE: 0.1% ADDED • REFRIGERATE**

Total Act.: <1.7 microcuries $^{125}$I As of Noon EST

LOT NO.: EXP. DATE:

E. R. Squibb & Sons, Inc.
Princeton, NJ 08540 Made in USA C6673/09421


## Angiotensin I IMMUTOPE®Kit

List 09421

For *in vitro* Diagnostic Use • **WARNING: NOT FOR INJECTION**

**Not for Internal or External Use in Humans or Animals**

Contains sufficient material for 200 tubes

**REFRIGERATE** • See insert

**E. R. Squibb & Sons, Inc.,** Princeton, NJ 08540

**Made in USA** C5149/09421

CONTENTS:

1 bottle (200 ml) $^{125}$I ANGIOTENSIN I BUFFER <1.7 microcuries per bottle

6 bottles (2 ml ea.) ANGIOTENSIN I STANDARD (1 each of 0, 50, 100, 200, 300, and 500 pg/25 μl, respectively)

1 vial (10 ml) ANGIOTENSIN I ANTISERUM

1 bottle (210 tabs.) ANGIOTENSIN I ADSORBENT CHARCOAL TABLETS

1 vial (5 ml) ANGIOTENSIN I PLASMA BUFFER (pH 7.4)

1 vial (2 ml) DIMERCAPROL SOLUTION

1 vial (330 mg) 8-HYDROXYQUINOLINE SULFATE

NEW ASSAY PROCEDURE

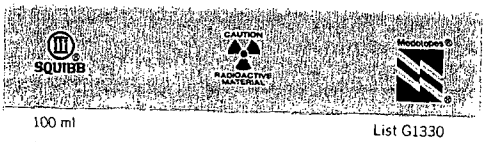
SQUIBB *IN VITRO*

# Angiotensin I IMMUTOPE® Kit

For Quantitative Measurement of Plasma Renin Activity by Radioimmunoassay

For *IN VITRO* Diagnostic Use
For Professional Use Only

06242




100 ml

List G1330

# $^{125}$I DIGOXIGENIN

For use with Digoxin CLASP® RIA Kit
For *in vitro* Diagnostic Use • See insert
**Not for Internal or External Use in Humans or Animals**
Store at 2° to 8° C • SODIUM AZIDE: 0.1%

Total Act.: < 7 microcuries $^{125}$I
LOT NO.:

As of EXP. DATE:

Noon EST

E. R. Squibb & Sons, Inc., Princeton, NJ 08540
Made in USA

C6648 / G1330


# Digoxin CLASP® RIA Kit

List G1330

Squibb Digoxin Radioimmunoassay Kit
For *in vitro* Diagnostic Use • **WARNING: NOT FOR INJECTION**
**Not for Internal or External Use In Humans or Animals**
Contains sufficient material for 100 tubes
**STORE AT 2° to 8° C • See insert**

**CONTENTS:**
1 bottle (100 ml) $^{125}$I DIGOXIGENIN
<7 microcuries $^{125}$I per bottle
6 bottles (1 ml ea.) DIGOXIN STANDARD
(1 each of 0, 0.5, 1.0, 2.0, 3.0 and 5.0 ng per ml)
1 bottle (1 ml) DIGOXIN CONTROL
DIGOXIN STANDARDS and CONTROL contain human serum

**E. R. Squibb & Sons, Inc., Princeton, NJ 08540**
Made in USA

C5146 / G1330

SQUIBB

J3-431C

Revised May 1980

# Digoxin CLASP® RIA Kit

## For Quantitative Measurement of Serum Digoxin Levels by Radioimmunoassay

## For *IN VITRO* Diagnostic Use
## For Professional Use Only

### DETERMINATION OF SERUM DIGOXIN LEVELS BY RADIOIMMUNOASSAY

Measurement of body constituents or administered compounds by the technique of radioimmunoassay offers a bioanalytical tool that combines the extreme sensitivity of radioisotope methodology with the extreme specificity of immunological techniques. The procedure requires a specific antibody, a radiolabeled antigen, a pure sample of the antigen to serve as a reference standard, and a means of separation of free antigen from antibody-bound antigen. The procedure follows the basic principle of saturation analysis, where there is competition between labeled and unlabeled antigen for a fixed number of antibody binding sites. As the concentration of unlabeled antigen (the substance actually being measured) increases, less of the added radiolabeled antigen will be bound to the antibody. After a specified period of time, the bound component is separated from the free component. The radioactivity in each bound fraction is then measured. The absolute quantity of unlabeled antigen in the sample being analyzed is determined by comparing the assay results to a standard curve prepared with known amounts of the unlabeled antigen.

In the Digoxin CLASP RIA Kit, antibody to digoxin (produced in New Zealand white rabbits by administration of digoxin coupled to a protein

### DIGOXIN CHEMICAL AND BIOLOGICAL PROPERTIES

**Chemical Properties:** Digoxin is a pure glycoside obtained from the leaves of *Digitalis lanata*. Like all cardiac glycosides, digoxin consists of a steroidal portion or aglycone, and a glycosidic portion, consisting of three digitoxose sugar residues. Digoxin is formed upon partial hydrolysis of the naturally occurring Lanatoside C found in *Digitalis lanata*. It differs from digitoxin by the presence of an extra hydroxyl group at the C-12 position. Because of this structural difference, digoxin shows increased polarity and decreased lipid solubility, resulting in a marked difference in the pharmacokinetics of the two compounds.

The pharmacologic activity of cardiac glycosides is contained exclusively in the steroidal (aglycone or genin) portion of the molecule. The sugars possess no intrinsic activity, but they enhance the pharmacologic activity of the aglycone several times, presumably by increasing solubility or enhancing the ability of the drug to penetrate cell membranes. The pharmacologically active aglycone portion of the digoxin molecule, devoid of the sugar residues, is referred to as digoxigenin, and is the radiolabeled component of the Digoxin CLASP RIA Kit.

**Biological Properties:** Digoxin is well absorbed from the gastrointestinal tract, with approximately 80 percent of an oral dose being even-

SQUIBB

CAUTION
RADIOACTIVE MATERIAL

Medotopes®

100 ml

List H0810

# ESTRIOL PREMIX

$^{125}$I Estriol and Separant Mixture
For *in vitro* Diagnostic Use • See insert
**Not for Internal or External Use in Humans or Animals**
**SODIUM AZIDE:** 0.06% added
**REFRIGERATE** • SHAKE WELL BEFORE USE

Total Act.: <8.6 microcuries $^{125}$I

As of Noon EST

LOT NO.:

EXP. DATE:

E. R. Squibb & Sons, Inc., Princeton, NJ 08540
Made in USA

C6723 / H0810

SQUIBB

CAUTION
RADIOACTIVE MATERIAL

Medotopes®

List H0810

# ESTRIOL-SQUIBB RADIOIMMUNOASSAY Kit

For *in vitro* Diagnostic Use
**WARNING: NOT FOR INJECTION**
**Not for Internal or External Use in Humans or Animals**
Contains sufficient material for 100 tubes
**REFRIGERATE** • See insert

CONTENTS:
1 bottle (100 ml) ESTRIOL PREMIX
<8.6 microcuries $^{125}$I per bottle
1 bottle (50 ml) ESTRIOL ANTISERUM
6 vials (1.2 ml ea.) ESTRIOL STANDARD (1 each of 0, 1, 4, 8, 16 and 32 ng per ml)

ESTRIOL STANDARDS contain human serum

E. R. Squibb & Sons, Inc., Princeton, NJ 08540 USA

C5152 / H0810

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OK for production
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SQUIBB®

E. R. Squibb & Sons, Inc.
Princeton, NJ 08540

Printed in USA

Issued October 1980

J3-486

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# ESTRIOL-SQUIBB RADIOIMMUNOASSAY KIT

For Quantitative Measurement of Unconjugated Estriol Levels in Serum by Radioimmunoassay

For *IN VITRO* Diagnostic Use

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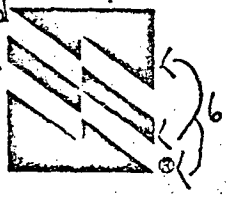

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# ESTRIOL-SQUIBB RADIOIMMUNOASSAY KIT

**For Quantitative Measurement of Unconjugated Estriol Levels in Serum by Radioimmunoassay**

**For *IN VITRO* Diagnostic Use**

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## Table of Contents





## FUNCTION

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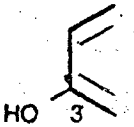

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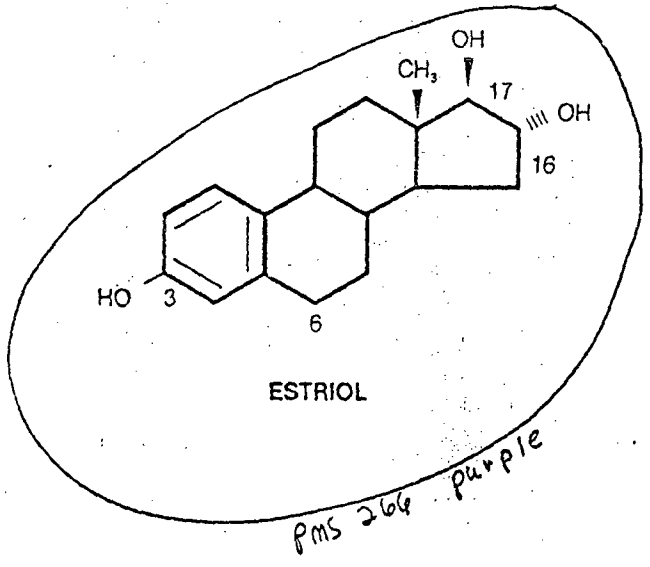
## FUNCTION OF THE TEST

Careful pregnancy management requires dependable methods of fetoplacental monitoring. This monitoring is especially important in such high-risk circumstances as chronic hypertension, preeclampsia, diabetes mellitus, intrauterine-growth retardation, postmaturity, cardiovascular disease, hormonal disorders, and in patients with a past history of problem pregnancies.[1,2] Direct methods for evaluation of fetal status require hospitalization and/or expensive equipment. The determination of unconjugated or "free" estriol in the maternal blood system is an inexpensive, indirect method for monitoring fetoplacental status, because consistently low levels of estriol or a sudden drop in estriol levels may be indicative of fetal distress.[1,3]




ESTRIOL

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Estriol begins to appear in the maternal blood system in the ninth week of gestation. By the end of the third trimester, the serum level of unconjugated estriol has risen to as high as 20 to 30 ng/ml. Virtually all of the estriol found in the maternal blood system is synthesized in the fetoplacental unit.[4] The fetal adrenals produce dehydroepiandrosterone sulfate (DHEAS) which is enzymatically hydroxylated at the 16α position by the fetal liver. This 16α OH-DHEAS is aromatized to estriol in the placenta and released into the maternal blood system. The half-life of free estriol in the maternal blood system is only 20 to 30 minutes[5-7] because the maternal liver conjugates estriol to a mixture of sulfates and glucuronides for facile urinary excretion. Because of this short half-life, changes in fetoplacental status are rapidly reflected.

Other assay procedures quantitate the amount of total estriol, free and conjugated, in the blood sample. However, the amount of conjugated estriol has been found to be sensitive to renal excretion.[8,9] Therefore, these procedures introduce unnecessary, complicating factors into the diagnostic accuracy of the method.

The ESTRIOL-SQUIBB RADIOIMMUNOASSAY KIT measures only free (unconjugated) estriol. The specificity of the antiserum included in the kit obviates the need for any pretreatment of the serum sample, thereby substantially reducing sample handling.

Estriol levels are expressed in nanograms (ng, or $10^{-9}$ g) of estriol per milliliter of serum (ng/ml).

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## PRINCIPLES OF THE TEST

The technique of radioimmunoassay offers a bioanalytical tool that combines the extreme sensitivity of radioisotope methodology with the extreme specificity of immunological techniques. The procedure requires a specific antibody, a radiolabeled antigen, a pure sample of the antigen to serve as a reference standard, and a means of separation of free antigen from antibody-bound antigen. The procedure follows the basic principle of saturation analysis, where there is competition between labeled and unlabeled antigen for a fixed number of antibody binding sites. As the concentration of unlabeled antigen (the substance actually being measured) increases, less of the radiolabeled antigen will be bound to the antibody. This competitive reaction is depicted in Figure I. After a specified period of time, the free and bound components of the mixture are separated, and the radioactivity of the bound components is measured. The absolute quantity of unlabeled antigen in the sample being analyzed is determined by comparing the assay result to a standard curve prepared with known amounts of the unlabeled antigen.

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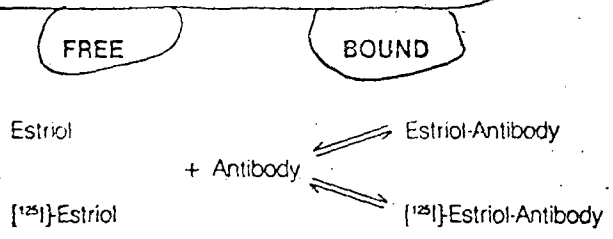
FIGURE I/The competitive binding reaction.

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In the ESTRIOL-SQUIBB RADIOIMMUNOASSAY KIT, antibody to estriol (produced in New Zealand white rabbits by administration of a derivative of estriol that was coupled at the 6 position to a protein carrier) is the specific antibody, a purified tyrosyl-containing estriol derivative iodinated with $^{125}I$ serves as the radiolabeled antigen, and purified estriol is the reference standard. Separation of the free and bound radiolabeled antigen is achieved by a double antibody accelerator system consisting of goat anti-rabbit gamma-globulin and polyethylene glycol. In order to reduce the number of pipetting steps involved in the assay procedure, the radiolabel and the separant have been combined into a single reagent.

The half-life of $^{125}I$ is 60.2 days. The isotope decays in a complex fashion with emission of x-rays and gamma rays whose radiation energies are 27.4 keV and 35.4 keV, respectively. These energies are well within the detection capability of modern solid crystal gamma counters. There is no beta emission.

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## REAGENTS

The ESTRIOL-SQUIBB RADIOIMMUNOASSAY KIT contains sufficient material for 100 tubes. Each kit contains the following components:

1. Estriol Antiserum: 50 ml, including less than 34 microliters of estriol antiserum per bottle, bovine serum albumin and sodium chloride (carriers), sodium barbital and tris(hydroxymethyl)-aminomethane (buffering agents), hydrochloric acid (pH adjustment), and sodium azide (a preservative) in water.
2. Estriol Premix ($^{125}$I Estriol and Separant Mixture): 100 ml, with a total radioactivity of less than 8.6 microcuries of $^{125}$I per bottle, in an aqueous matrix containing goat anti-rabbit gamma-globulin (the second antibody), polyethylene glycol 4000 (the accelerator), normal rabbit serum, bovine serum albumin, and sodium chloride (carriers), sodium barbital and tris(hydroxymethyl)-aminomethane (buffering agents), hydrochloric acid (pH adjustment), and sodium azide (a preservative).
3. Estriol Standards: six vials, 1.2 ml each, containing 0, 1, 4, 8, 16, and 32 ng of estriol per ml in a matrix of human serum including sodium azide (a preservative) and a trace quantity of ethanol (a solubilizer).

**THE ESTRIOL PREMIX MUST BE SHAKEN WELL IMMEDIATELY BEFORE USE.**

Reagents within each kit are specific to the lot number on the kit; therefore, only reagents from kits with the same lot number may be used interchangeably.

All reagents and samples must be equilibrated to room temperature before use.

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## Warnings

For *In Vitro* Diagnostic Use.

Restricted Device: Federal law restricts the sale, distribution, or use of this device to, by, or on the lawful order of, a health professional.

Note: This radioactive material may be received, acquired, possessed, and used only by physicians, veterinarians in the practice of veterinary medicine, clinical laboratories or hospitals and only for in vitro clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use, and transfer are subject to the regulations and a general license of the US Nuclear Regulatory Commission or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority.

E.R. Squibb & Sons, Inc.

In vitro clinical laboratory testing with the ESTRIOL-SQUIBB RADIOIMMUNOASSAY KIT requires only a general license from the US Nuclear Regulatory Commission. The general license is issued to any physician, clinical laboratory, or hospital obtaining a validated registered USNRC Form 483. This form must be submitted in triplicate to the USNRC. The possessor of a general license is subject to the conditions and limitations under 10 CFR 31.11. (A specific license is available from the USNRC for quantities larger than 200 microcuries.

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## Precautions

Observe the following safety rules in handling radioactive material:

- There should be no pipetting by mouth, smoking, or consumption of any food or drink in areas where radioactive materials are permitted.
- Wear gloves when handling radioactive materials.
- Wash hands thoroughly after handling radioactive materials.
- Working areas should be covered with disposable absorbent paper.
- Wipe up all spills quickly and thoroughly and discard the contaminated materials with the radioactive waste.
- Solid waste may be stored in a specifically designated area in a covered metal or plastic container that has been identified with a USNRC radiation caution label until the waste decays to a safe level. Then, after defacing the labeling, the material may be discarded with nonradioactive waste.
- The user should determine whether USNRC and/or local regulations permit the liquid radioactive waste to be discarded through the sanitary sewerage system.

Because Estriol Standards contain human serum, these reagents, and patient samples, should be handled in the same manner as any potentially infective biological material. The human serum in the standards has been found to be nonreactive for $HB_SAg$ when tested with licensed reagents.

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## Chemical Hazard

- All of the reagents in this kit contain the commonly used bacteriostatic agent, sodium azide. Disposal of these materials through the laboratory plumbing system could lead to the formation of highly explosive copper and lead azides. In order to reduce the possibility of an explosion, it is recommended that large amounts of water be used to flush excess reagents through the plumbing system.

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## Storage

The kit should be refrigerated upon receipt.

**All reagents should be stored in their original containers, with their original closures. Do not transfer these reagents to other containers or change the closures for storage purposes.**

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## SPECIMEN COLLECTION AND PREPARATION

An appropriate quantity of blood should be collected from a peripheral vein using a standard blood collection tube that does not contain an anticoagulant. The patient does not need to be in a fasting state when the blood is withdrawn. In order to avoid the observation of spurious changes in estriol values, the position of the patient during blood collection and the method of blood collection should be standardized.

Serum, not plasma, should be used in performing the test. The variables introduced into the procedure through the use of plasma could make the results unreliable.

It is preferable to separate the serum sample on the same day that the blood is withdrawn from the patient. Serum samples may be stored in a refrigerator for up to 24 hours, and should be frozen if stored for longer periods. Frozen samples should be equilibrated to room temperature and mixed well before use. Samples should not be refrozen.

The use of hemolyzed or lipemic samples is not recommended.

The patient's history should be thoroughly scrutinized to determine if any diagnostic or therapeutic radioisotopes have been administered within the week or two immediately prior to the estriol determination. If the patient has been treated with radioisotopes or if the patient's history is unavailable, the activity of the serum sample should be checked in a scintillation well counter that is set for $^{125}I$. If after a few seconds it appears that the count rate is above background, 20 microliters of the serum sample should be accurately counted to determine whether the radioactivity contained in this volume could significantly affect assay results.

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## TEST PROCEDURE

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### Materials Needed

In addition to the reagents supplied with the ESTRIOL-SQUIBB RADIOIMMUNOASSAY KIT, the following materials are required:

- 12 x 75 mm plastic test tubes
- Centrifuge capable of 3000 to 4000 rpm
- Well-type gamma scintillation counter (discriminator settings of 20 to 50 keV)
- Refrigerator
- Vortex mixer
- Pipettor and tips for 50 microliters (μl) [and, optionally 500 μl]
- Repeating pipettors capable of delivering 1 ml and 500 μl
- Container for radioactive waste
- Test tube racks
- Controlled-temperature water bath (37° C)

### Procedural Precautions

Good laboratory practice dictates that an appropriate number of controls be analyzed in conjunction with each set of samples.

All reagents and samples must be equilibrated to room temperature before use.

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| 7 and |
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| 11 and |
| 13 and |
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*If desired
follows:
a) Mark t
b) Add 50
c) Add 50
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## Procedure

The technique described below is based upon the performance of duplicate analyses for all standards, samples, and controls. Read the entire procedure before starting the test.

1. Mark a series of 12 x 75 mm plastic test tubes with numerals 1 through 14. The first two tubes are used for measuring total counts and the remaining 12 tubes are required for generation of a standard curve. Two additional tubes will be needed for each clinical sample or control to be assayed.*
2. Add 50 µl of Estriol Standard or 50 µl of the clinical sample to the respective tubes as follows:

| Tubes | Sample to be Added |
|---|---|
| 1 and 2 | None |
| 3 and 4 | 50 µl of 0 ng/ml Standard |
| 5 and 6 | 50 µl of 1 ng/ml Standard |
| 7 and 8 | 50 µl of 4 ng/ml Standard |
| 9 and 10 | 50 µl of 8 ng/ml Standard |
| 11 and 12 | 50 µl of 16 ng/ml Standard |
| 13 and 14 | 50 µl of 32 ng/ml Standard |
| 15 and 16 | 50 µl of Clinical Sample |

*If desired, nonspecific binding can be determined as follows:

a) Mark two tubes as NSB.
b) Add 50 µl of "0" Standard to each tube.
c) Add 500 µl of distilled water to each tube.
d) Treat these two tubes in the same manner as tubes 3 through 14 by completing steps 4 through 10.

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3. Add 500 µl of Estriol Antiserum to tubes 3 through 14.
4. Add 1.0 ml of Estriol Premix to each tube. **THIS REAGENT MUST BE SHAKEN WELL IMMEDIATELY BEFORE USE.**
5. Vortex each tube.
6. Set tubes 1 and 2 aside and incubate the remaining tubes in a controlled-temperature water bath at 37° C for 45 minutes.
7. Centrifuge the incubated tubes for 5 to 10 minutes at 3000 to 4000 rpm.
8. Decant the supernatant by gently inverting each tube once, discarding the liquid into a radioactive waste container. Keeping the tube inverted, touch the rim on an absorbent paper and gently tap the tube.
9. Measure the radioactivity in all tubes in a standard well-type gamma scintillation counter (discriminator settings of 20 to 50 keV) for one minute or for a fixed amount of time that is sufficient to eliminate counting statistics as an important source of variability.
10. Subtract background cpm and record net cpm for each tube.

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## RESULTS

1. It is possible to plot either % Bound or B/Bo or net cpm versus concentration on linear-linear graph paper. Use the following formula to calculate % Bound for each tube and record the results on a worksheet.

$$\% \text{ Bound} = \frac{\text{PELLET COUNTS (net cpm)} \times 100}{\text{TOTAL COUNTS (net cpm)}}$$

Example:

Background cpm: 150
Tube #3 PELLET COUNTS (gross cpm): 52,398
TOTAL COUNTS (gross cpm): 98,227

$$\% \text{ Bound} = \frac{52{,}398 - 150}{98{,}227 - 150} \times 100$$

$$= \frac{52{,}248}{98{,}077} \times 100 = 53.3$$

Alternatively, use the following formula to calculate B/Bo values for each tube and record the results on a worksheet.

$$\text{B/Bo} = \frac{\text{PELLET COUNTS (net cpm)}}{\text{Avg. PELLET COUNTS of "0" Std. (net cpm)}}$$

Example:

Background cpm: 150
Patient #1 PELLET COUNTS (gross cpm): 25,150
"0" Std. - Avg. PELLET COUNTS
(gross cpm): 50,150

$$\text{B/Bo} = \frac{25{,}150 - 150}{50{,}150 - 150}$$

$$= \frac{25{,}000}{50{,}000} = 0.50$$

2. Prepare a standard curve by plotting either % Bound or B/Bo or net cpm against the concentrations of the standards. A typical set of data calculated in terms of % Bound is given in Table I and graphed in Figure II. The same set of data calculated in terms of B/Bo is listed in Table II and plotted in Figure III. These curves are provided for guidance only and **should not** be used in calculating the estriol levels in the clinical samples.
3. Determine the concentration (ng/ml) of estriol in each sample by referring to the standard curve prepared earlier. The observed % Bound or B/Bo or net cpm for each sample will correspond to a specific estriol concentration. Samples with estriol concentrations of greater than 32 ng/ml should be accurately diluted with "0" standard and reassayed, if an exact value is required.

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**TABLE I (Typi
Terms of % I**

| TOTAL COUNT | |
|---|---|
| | ...dan |
| Concentration | |
| 0 | |
| 0 | |
| 1 | |
| 1 | |
| 4 | |
| 4 | |
| 8 | |
| 8 | |
| 16 | |
| 16 | |
| 32 | |
| 32 | |

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TABLE I/Typical Standard Curve Data Calculated in Terms of % Bound

| TOTAL COUNT | | | 94,683 net cpm |
|---|---|---|---|
| Standard Concentration (ng/dl) | Pellet Counts (net cpm) | % Bound | Avg. % Bound |
| 0 | 47,080 | 49.7 | |
| 0 | 46,169 | 48.8 | 49.2 |
| 1 | 42,694 | 45.1 | |
| 1 | 41,739 | 44.1 | 44.6 |
| 4 | 34,254 | 36.2 | |
| 4 | 34,039 | 36.0 | 36.1 |
| 8 | 28,752 | 30.4 | |
| 8 | 26,770 | 30.4 | 30.4 |
| 16 | 23,900 | 25.2 | |
| 16 | 23,848 | 25.2 | 25.2 |
| 32 | 19,781 | 20.9 | |
| 32 | 19,894 | 21.0 | 21.0 |

19

FIGURE II/A graph of % Bound vs. Estriol Concentration for a typical standard curve obtained with the ESTRIOL-SQUIBB RADIOIMMUNOASSAY KIT. As indicated, a patient sample having a mean percent bound of 27.5 would have an estriol concentration of 11.4 ng/ml.

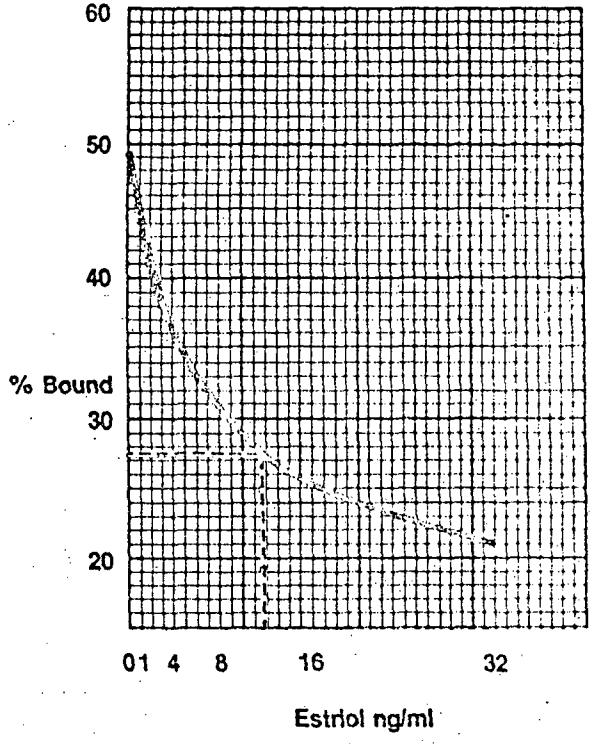


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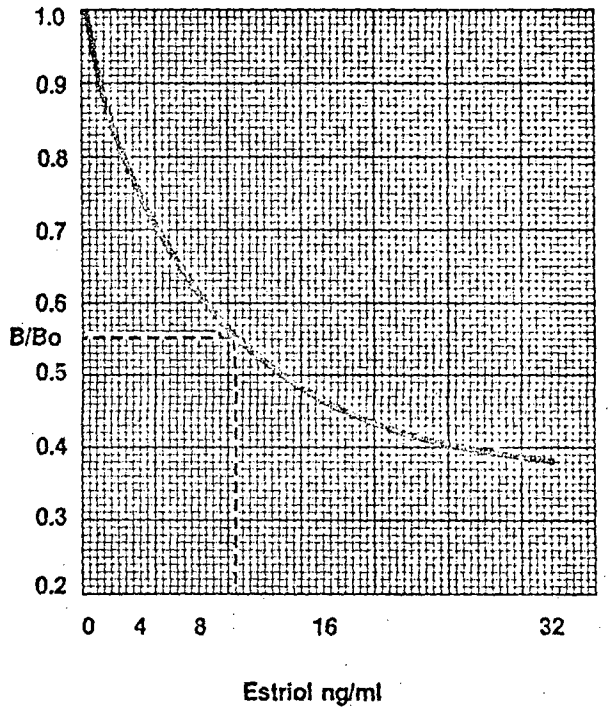
TABLE II/Typical Standard Curve Data Calculated in Terms of B/Bo

| TOTAL COUNT | | | 94,683 net cpm |
|---|---|---|---|
| Bo Counts (mean avg.) | | | 46,624 cpm |
| Standard Concentration (ng/ml) | Pellet Counts (net cpm) | B/Bo | Avg. B/Bo |
| 0 | 47,080 | 1.01 | |
| 0 | 46,169 | 0.99 | 1.00 |
| 1 | 42,694 | 0.91 | |
| 1 | 41,739 | 0.89 | 0.90 |
| 4 | 34,254 | 0.72 | |
| 4 | 34,039 | 0.72 | 0.72 |
| 8 | 28,752 | 0.60 | |
| 8 | 28,770 | 0.60 | 0.60 |
| 16 | 23,900 | 0.49 | |
| 16 | 23,848 | 0.48 | 0.48 |
| 32 | 19,781 | 0.39 | |
| 32 | 19,894 | 0.40 | 0.39 |



FIGURE III/A graph of B/Bo vs. Estriol Concentration for a typical standard curve obtained with the ESTRIOL-SQUIBB RADIOIMMUNOASSAY KIT. As indicated, a patient sample having a mean B/Bo of 0.55 would have an estriol concentration of 10.4 ng/ml.

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## LIMITATIONS OF THE TEST PROCEDURE

Because the ranges for normal and abnormal levels of unconjugated estriol overlap, it is important to establish a trend for each patient through periodic sampling. The frequency of sampling will depend upon the type and severity of the particular problem. Unconjugated estriol levels have been observed to drop precipitously from one measurement to the next without apparent pathologic cause.[1] One group of investigators[10] suggests that a single determination must be at least 40 to 45 percent less than the mean of the previous three determinations in order to be more than 99 percent confident that the drop is indicative of fetal distress. Observed unconjugated estriol values of less than 3 ng/ml in the last 12 weeks of pregnancy and less than 4 ng/ml in the last six weeks of pregnancy are highly correlated with fetal distress.[6] Because there have been numerous cases [2,11-14] in which normal estriol levels were not indicative of fetal distress and other examples [14,15] of women with low estriol levels who gave birth to vigorous, healthy neonates, medical intervention should be based upon the patient's entire clinical picture. Other diagnostic procedures such as fetal heart monitoring, amniocentesis, ultrasonography and stress testing may be used to confirm estriol results.



## EXPECTED VALUES

A total of 119 serum samples from women in the ninth to forty-second week of gestation were assayed. The results are summarized in Table III. Eight of the nine samples from women in the ninth to thirteenth week of gestation were found to contain less than 1 ng of estriol per ml. The individual data points were utilized to compute 95 percent confidence intervals for **predicted** estriol levels. Because the highest standard included in the ESTRIOL-SQUIBB RADIOIMMUNOASSAY KIT contains 32 ng of estriol per ml, any sample having a larger value is reported as being ">32.00". For the purpose of this analysis, these samples were assigned a value of 32. Therefore, those predictions, which are listed in Table III and graphically portrayed in Figure IV, are conservative.

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TABLE III/Normal Range Data*

| Weeks Gestation | Number of Samples | Range (ng/ml) | Mean (ng/ml) | Predicted Levels (ng/ml) | 95% Confidence Limits Lower (ng/ml) | 95% Confidence Limits Upper (ng/ml) |
|---|---|---|---|---|---|---|
| 9 | 2 | <1.0 | <1.0 | — | — | — |
| 10 | 2 | <1.0 | <1.0 | — | — | — |
| 12 | 2 | <1.0–2.3 | 1.6 | — | — | — |
| 13 | 3 | <1.0 | <1.0 | — | — | — |
| 16 | 1 | 5.0 | 5.0 | 1.9 | 0.8 | 4.4 |
| 20 | 3 | 3.1–4.4 | 3.8 | 2.7 | 1.2 | 6.2 |
| 21 | 1 | 2.9 | 2.9 | 3.0 | 1.3 | 0.8 |
| 22 | 4 | 2.9–4.4 | 3.7 | 3.3 | 1.4 | 7.4 |
| 23 | 1 | 2.4 | 2.4 | 3.6 | 1.6 | 8.1 |
| 24 | 3 | 2.2–4.4 | 3.0 | 3.9 | 1.7 | 8.9 |
| 25 | 3 | 2.2–5.9 | 3.7 | 4.3 | 1.9 | 9.7 |
| 26 | 3 | 2.8–5.9 | 4.7 | 4.7 | 2.1 | 10.6 |
| 27 | 1 | 4.5 | 4.5 | 5.1 | 2.3 | 11.6 |
| 28 | 2 | 4.5–4.6 | 4.6 | 5.6 | 2.5 | 12.6 |
| 29 | 2 | 4.6–5.4 | 5.0 | 6.1 | 2.7 | 13.8 |
| 30 | 5 | 3.8–8.9 | 6.3 | 6.7 | 3.0 | 15.1 |
| 31 | 2 | 4.9–6.0 | 5.4 | 7.3 | 3.3 | 16.5 |
| 32 | 2 | 12.1–13.5 | 12.8 | 8.0 | 3.6 | 18.0 |
| 34 | 9 | 4.4–13.2 | 7.9 | 9.6 | 4.3 | 21.6 |
| 35 | 15 | 7.3–17.8 | 10.5 | 10.5 | 4.7 | 23.6 |
| 36 | 8 | 6.7–25.0 | 13.2 | 11.5 | 5.1 | 25.9 |
| 37 | 7(1)* | 9.4–>32.0 | 19.2 | 12.6 | 5.6 | 28.3 |
| 38 | 9 | 9.7–30.6 | 18.5 | 13.8 | 6.1 | 31.0 |
| 39 | 11(1)* | 11.8–>32.0 | 18.8 | 15.1 | 6.7 | 33.9 |
| 40 | 8(1)* | 7.1–>32.0 | 16.6 | 16.5 | 7.3 | 37.2 |
| 41 | 7 | 7.2–27.1 | 16.1 | 18.0 | 8.0 | 40.7 |
| 42 | 3 | 7.8–25.5 | 17.6 | 19.7 | 8.7 | 44.6 |

*Samples containing less than 1 ng or greater than 32 ng of estriol per ml are reported as "<1.0" or ">32.0", respectively. The limit values were used to calculate the results appearing in this table.

* Number of samples with a value of >32.0.

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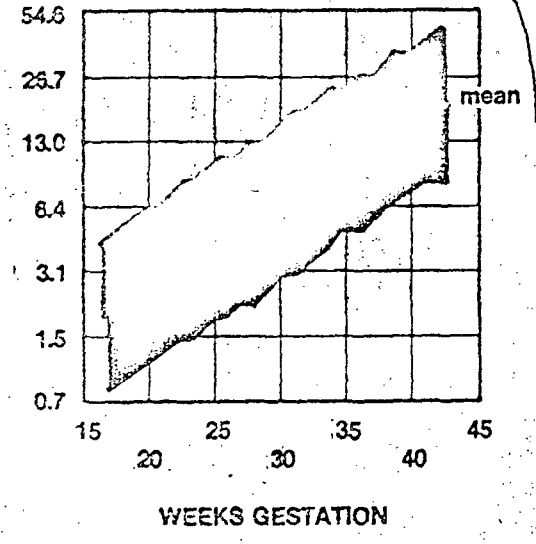
FIGURE IV/Expected range of estriol levels as measured by the ESTRIOL-SQUIBB RADIOIMMUNOASSAY KIT as a function of time of gestation. The mean and the 95% confidence limits are shown. (see the footnotes in Table III.)

FIGURE V/Regression analysis of 110 samples analyzed by the ESTRIOL-SQUIBB RADIOIMMUNOASSAY KIT and by the NEN method. The limit values of 1.0, 32.0, and 36.0 were used to calculate the results appearing in this figure.

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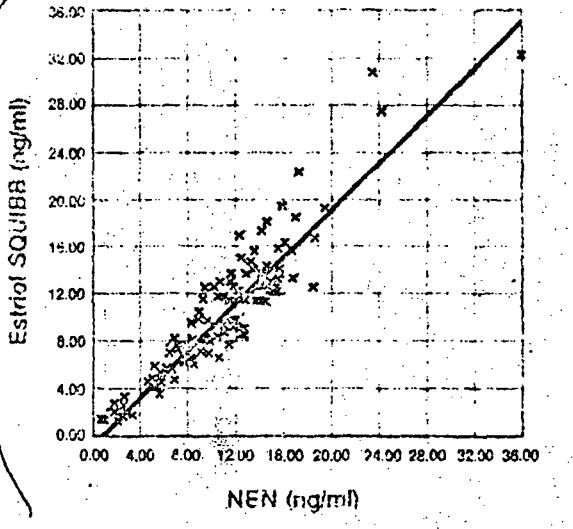


In another study the sera of 110 pregnant women were analyzed by the ESTRIOL-SQUIBB RADIOIMMUNOASSAY KIT and by the RIANEN™ Estriol [$^{125}$I] Radioimmunoassay Kit sold by New England Nuclear. The regression analysis of these data is shown in Figure V.

| | Estriol-SQUIBB | NEN |
|---|---|---|
| Range (ng/ml) | 1.0–32.0 | 1.0–36.0 |
| Mean (ng/ml) | 10.18 | 10.99 |
| Correlation Coefficient | 0.94 | |
| Intercept | −0.64 | |
| Slope | 1.00 | |

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## SPECIFIC PERFORMANCE CHARACTERISTICS

### Standard Curve

Typical standard curves are shown in Figures II and III (see pages 19 and 21).

### Sensitivity

The lowest estriol-containing standard supplied with the kit contains 1 ng/ml.

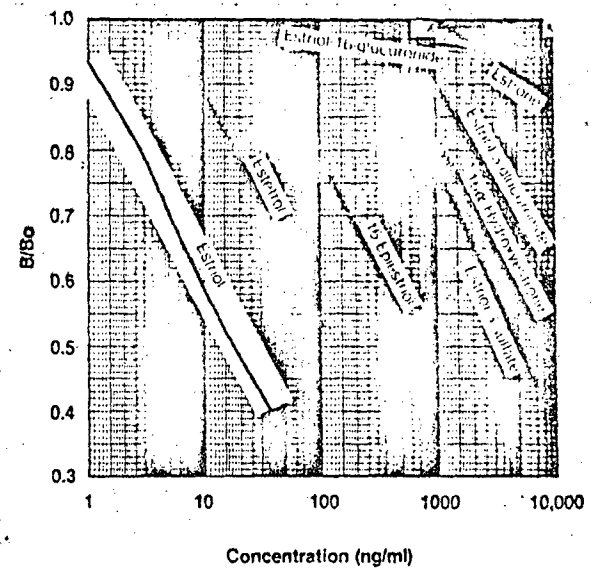
### Specificity

The cross-reactivity of the antiserum with various steroids is depicted in Figure VI and tabulated in Table IV.

The relatively large cross-reactivity of the antiserum with 16-epiestriol and estetrol will be of little practical consequence because serum levels of these steroids are very low.[16,17]



**FIGURE VI/Cross-reactivity of various steroids in the ESTRIOL-SQUIBB RADIOIMMUNOASSAY KIT.**




x———x Aldosterone, Cholesterol, Corticosterone, Cortisol, Cortisone, 11-Deoxycortisol, Dehydroepiandrosterone, Estradiol, 17α-Hydroxyprogesterone, Progesterone, and Testosterone



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TABLE IV/Cross-Reactivity of Various Steroids with the ESTRIOL-SQUIBB RADIOIMMUNOASSAY KIT

| Steroid | Percent Cross-Reactivity at a Concentration of 10 μg/ml |
|---|---|
| Aldosterone | < 0.01 |
| Cholesterol | < 0.01 |
| Corticosterone | < 0.01 |
| Cortisol | < 0.01 |
| Cortisone | < 0.01 |
| 11-Deoxycortisol | < 0.01 |
| Dehydroepiandrosterone | < 0.01 |
| 16-Epiestriol | 3.33* |
| Estetrol | 13.73* |
| Estradiol | < 0.01 |
| Estriol-3-glucuronide | 0.18 |
| Estriol-16-glucuronide | 0.02 |
| Estriol-3-sulfate | 0.40 |
| Estrone | 0.01 |
| 16α-Hydroxyestrone | 0.10** |
| 17α-Hydroxyprogestrone | < 0.01 |
| Progestrone | < 0.01 |
| Testosterone | < 0.01 |

*Percent cross-reactivity at a concentration of 0.1 μg/ml

**Percent cross-reactivity at a concentration of 1 μg/ml



## Recovery Efficiency

An important criterion of assay validity is the recovery of exogenous estriol from serum samples. Various amounts of exogenous estriol were added to a serum pool composed of samples taken from normal pregnant women having an endogenous estriol content of 2.49 ng/ml. Each samples was then assayed a total of 12 times over three separate runs. The results, which are presented in Table V, demonstrate quantitative recovery of the exogenous estriol.

TABLE V/Recovery Efficiency

| Endogenous Concentration (ng/ml) | Amount Added (ng/ml) | Total Expected (ng/ml) | Total Found (ng/ml) | Percent Recovery* |
|---|---|---|---|---|
| 2.49 | 2.50 | 4.95 | 5.38 | 108.7 |
| 2.49 | 5.00 | 7.49 | 7.45 | 99.5 |
| 2.49 | 10.00 | 12.49 | 12.05 | 96.5 |
| 2.49 | 20.00 | 22.49 | 23.03 | 102.4 |

$$*\text{Percent Recovery} = \frac{\text{Total Found}}{\text{Total Expected}} \times 100$$

## Parallelism

This type of study indicates whether patient samples and standards behave similarly toward the radiolabel and the antiserum, thereby validating the matrix of the standards and the use of the zero standard as a diluent for patient samples. A serum sample having a known analyte concentration is serially diluted with zero standard and the original sample and its dilutions are assayed. A plot of observed dose against expected dose should theoretically yield a 45° line through the origin. Such a study was performed with three separate samples containing high levels of estriol. The results, depicted in Figure VII, demonstrate the validity of the matrix for the standards and the use of the zero standard as a diluent.

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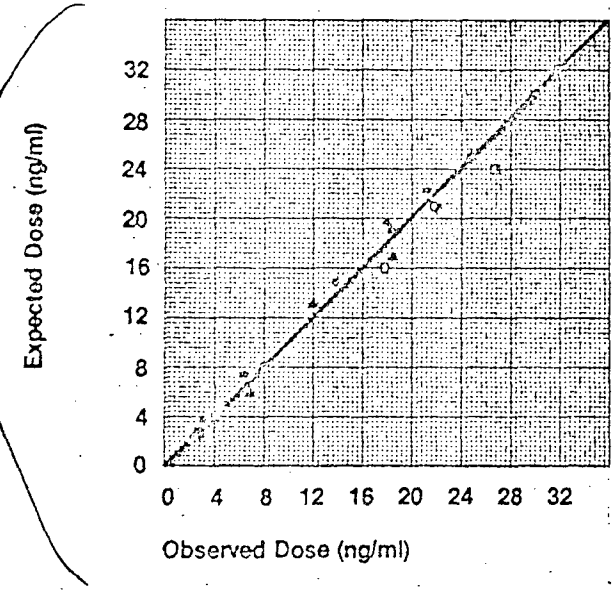
FIGURE VII/The parallelism study: A graph of observed dose versus expected dose. The theoretical 45° line is shown.




| Estriol - SQUIBB | Sample 1 (•) | Sample 2 (o) | Sample 3 (▲) |
|---|---|---|---|
| Slope | | | |
| Correlation | 1.059 | 0.926 | 0.966 |
| Intercept | 0.22 | 0.40 | 0.12 |
| Correlation Coefficient | 0.999 | 0.997 | 0.993 |



## Precision

Intra-assay CV data were obtained by performing a single run that consisted of 20 consecutive tubes each of human serum pools containing low, medium and high concentrations of estriol. In order to obtain inter-assay CV data, each of these controls was also assayed in duplicate in 12 runs done over a period of two months. The observed results yielded the reproducibility data shown in Table VI.

**TABLE VI/Precision Data**

| | Serum Pool | | |
|---|---|---|---|
| Parameter | Low | Medium | High |
| INTRA-ASSAY | | | |
| Mean (ng/ml) | 5.19 | 10.06 | 22.16 |
| Std. Dev. (ng/ml) | 0.36 | 0.89 | 1.46 |
| Range (ng/ml) | 4.58–5.68 | 8.53–11.65 | 18.75–23.83 |
| Coefficient of Variation (%) | 7.0 | 8.8 | 6.6 |
| INTER-ASSAY | | | |
| Mean (ng/ml) | 5.27 | 10.53 | 20.53 |
| Std. Dev. (ng/ml) | 0.53 | 0.90 | 1.93 |
| Range (ng/ml) | 4.34–6.43 | 8.64–12.08 | 17.49–25.18 |
| Coefficient of Variation (%) | 10.0 | 8.5 | 9.4 |

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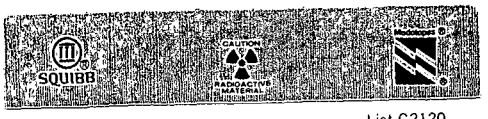



List G2120

50 ml

# $^{125}$I HTSH REAGENT

For use with HTSH CLASP® RIA Kit
For *in vitro* Diagnostic Use • See directions
Not for Internal or External Use in Humans or Animals
SODIUM AZIDE: 0.1%
STORE BELOW −10° C • SHAKE WELL BEFORE USE

Total Act.: <3 microcuries $^{125}$ As of Noon EST

LOT NO.: EXP. DATE:

E. R. Squibb & Sons, Inc., Princeton, NJ 08540
Made in USA

C6318B / G2120


List G2120

# HTSH CLASP® RIA Kit

Squibb HTSH Radioimmunoassay Kit
For *in vitro* Diagnostic Use • See enclosed directions
**Not for Internal or External Use in Humans or Animals**
Contains sufficient material for 100 tubes
**STORE BELOW −10°C**

CONTENTS:
1 bottle (50 ml) $^{125}$I HTSH REAGENT
<3 microcuries/bottle
1 bottle (4 ml) HTSH RIA STANDARD
0 μIU/ml
5 bottles (2 ml ea.) HTSH RIA STANDARD
(1 each of 2.5, 5.0, 10.0, 25.0 and 50.0 μIU/ml)
1 bottle (20 ml) HTSH ANTISERUM

E. R. Squibb & Sons, Inc., Princeton, NJ 08540
Made in USA

M5370A / G2120

SQUIBB

J3-445B

Revised June 1979

# HTSH CLASP® RIA Kit

**For Quantitative Measurement of Serum Human Thyroid Stimulating Hormone (HTSH) Levels by Radioimmunoassay**

**For *IN VITRO* Diagnostic Use**
**For Professional Use Only**

CAUTION
RADIOACTIVE MATERIAL
REFRIGERATE AT 2° to 8° C
Mediscopes ®

50 ml List 09026

# THYROSTAT®-3 LIOTHYRONINE I 125 BUFFER SOLUTION

0.03 microcurie or less per ml • SODIUM AZIDE: 0.1%
FOR LABORATORY USE ONLY with THYROSTAT-3
Adsorbent Tablets • See accompanying directions
For *in vitro* Diagnostic Use • **Shake gently before use**
**Not for Internal or External Use in Humans or Animals**

| | | |
|---|---|---|
| Total Act. <1.9 microcuries I 125 | As of | Noon EST |
| LOT NO. | EXP. DATE | |

E. R. Squibb & Sons, Inc.
Princeton, NJ 08540 Made in USA C6033B / 09026

SQUIBB

25 TEST PKG.

# THYROSTAT®-3 DIAGNOSTIC TEST KIT

For *in vitro* Diagnostic Use
For Evaluation of Thyroid Function
FOR LABORATORY USE ONLY
**Not for Internal or External Use in Humans or Animals**
**REFRIGERATE AT 2° to 8°C**

CONTENTS: LIST 09026
1 vial (28 tabs.) THYROSTAT-3 ADSORBENT TABLETS
1 bottle (50 ml) THYROSTAT-3 LIOTHYRONINE I 125 BUFFER SOLUTION <1.9 microcuries per bottle

**NOTE:** Buffer solution in this carton must be used with the accompanying Adsorbent Tablets.
See accompanying directions

E. R. Squibb & Sons, Inc.
Princeton, NJ 08540 Made in USA C6043A/09026

LOT NO.:
EXP. DATE:

REFRIGERATE AT 2° to 8° C

THYROSTAT®-3 DIAGNOSTIC TEST KIT

SQUIBB

CAUTION
RADIOACTIVE MATERIAL

25 TEST PKG.

List 09026

# THYROSTAT®-3 DIAGNOSTIC TEST KIT

For *in vitro* Diagnostic Use

For Evaluation of Thyroid Function

FOR LABORATORY USE ONLY • See accompanying directions

**Not for Internal or External Use in Humans or Animals**

CONTENTS:
1 vial (28 tabs.) THYROSTAT-3 ADSORBENT TABLETS
1 bottle (50 ml) THYROSTAT-3 LIOTHYRONINE I 125 BUFFER SOLUTION
<1.9 microcuries per bottle

**Note:** Buffer solution in this carton must be used with the accompanying Adsorbent Tablets.

REFRIGERATE AT 2° to 8° C

Medotopes®

THYROSTAT®-3 DIAGNOSTIC TEST KIT

06242


200 ml List 09028

**THYROSTAT®-3 LIOTHYRONINE I 125 BUFFER SOLUTION**

0.03 microcurie or less per ml • **SODIUM AZIDE: 0.1%**

FOR LABORATORY USE ONLY with THYROSTAT-3 Adsorbent Tablets • See accompanying directions.

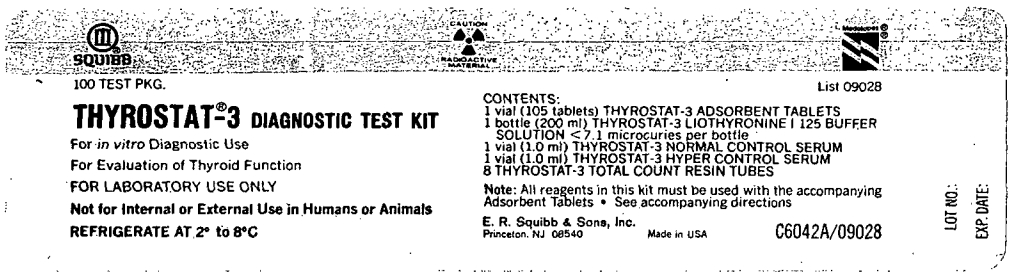
For *in vitro* Diagnostic Use • **Shake gently before use**

**Not for Internal or External Use in Humans or Animals**

Total Act. <7.1 microcuries I 125 As of Noon EST

LOT NO. EXP. DATE

E. R. Squibb & Sons, Inc.
Princeton, NJ 08540 Made in USA C6032B / 09028




100 TEST PKG. List 09028

**THYROSTAT®-3 DIAGNOSTIC TEST KIT**

For *in vitro* Diagnostic Use

For Evaluation of Thyroid Function

FOR LABORATORY USE ONLY

**Not for Internal or External Use in Humans or Animals**

**REFRIGERATE AT 2° to 8°C**

CONTENTS:
1 vial (105 tablets) THYROSTAT-3 ADSORBENT TABLETS
1 bottle (200 ml) THYROSTAT-3 LIOTHYRONINE I 125 BUFFER SOLUTION <7.1 microcuries per bottle
1 vial (1.0 ml) THYROSTAT-3 NORMAL CONTROL SERUM
1 vial (1.0 ml) THYROSTAT-3 HYPER CONTROL SERUM
8 THYROSTAT-3 TOTAL COUNT RESIN TUBES

**Note:** All reagents in this kit must be used with the accompanying Adsorbent Tablets • See accompanying directions

E. R. Squibb & Sons, Inc.
Princeton, NJ 08540 Made in USA C6042A/09028

LOT NO.:
EXP. DATE:

SQUIBB

J3-358B

# THYROSTAT®-3 DIAGNOSTIC TEST KIT

Revised February 1980

**For Quantitative Measurement of Serum Liothyronine ($T_3$) Uptake for the Evaluation of Thyroid Function**

**For *IN VITRO* Diagnostic Use**
**For Professional Use Only**

## PRINCIPLES OF THE TEST

The currently accepted principles underlying the $T_3$ (synonymous with liothyronine and triiodothyronine) uptake test are as follows:

The circulating thyroid hormones produced by the thyroid gland are bound to specific plasma proteins known collectively as thyroxine-binding proteins (TBP), and are in equilibrium with a small fraction of the free thyroid hormones circulating in the plasma. A change in the number of unoccupied TBP binding sites will alter the free thyroid hormonal level in the blood, which can be indirectly measured through the use of the $T_3$ uptake test.

In the $T_3$ uptake test, a supply of labeled exogenous thyroid hormone (in the Liothyronine I 125 Buffer Solution) is added to the patient's serum together with a secondary binding site (the Thyrostat-3 Adsorbent Tablet). A portion of the liothyronine $^{125}I$ will become bound to the binding sites of the TBP that are not occupied by the thyroxine whereas some, not bound to the TBP, will become bound to the adsorbent.

When the thyroid gland produces an excess of thyroid hormones, as in hyperthyroidism, the number of unoccupied TBP binding sites is reduced and a greater proportion of the added hormone will become bound to the adsorbent. Conversely, when thyroid hormone production is decreased, as in hypothyroidism, the number of unoccupied TBP binding sites is increased and a greater proportion of the added liothyronine $^{125}I$ will become bound to the TBP resulting in a decreased uptake by the adsorbent. (In normal pregnancy, although the free thyroid hormone level is normal, there is an increased production of TBP, so that more binding sites are available resulting in the binding of a greater proportion of the added liothyronine $^{125}I$ to these sites as in hypothyroidism.)

Therefore, the use of the $T_3$ uptake test provides an estimate of the unoccupied binding sites of the TBP in a given serum sample. This, in turn, gives an indirect estimate of the amount of endogenous circulating thyroid hormone, and therefore an indirect but reliable indication of thyroid function.

In summary, a large $T_3$ uptake indicates hyperthyroidism, while a small $T_3$ uptake indicates hypothyroidism (or normal pregnancy).

## RATIONALE FOR USE

The $T_3$ uptake test represents a significant advance in the search for a simple and reliable test of thyroid function. The test is an *in vitro* procedure which avoids any exposure of the patient to ionizing radiation. Equally important is the fact that the test is diagnostically significant in the presence of unrelated nonthyroidal factors which are known to complicate interpretation of other thyroid function tests. Although other thyroid function tests may be affected for considerable periods of time by the prior administration of most iodine-containing preparations, the $T_3$ uptake test is not so affected at the normal dose level at which these drugs are used. Anxiety, hypertension, congestive heart failure, or administration of mercurial agents also have no effect on the test.

The technique readily falls within the scope of any hospital or office laboratory with ordinary isotope facilities and is simple, rapid, and inexpensive enough to be used as a general screening test. Moreover, the test is consistently reliable when repeated at frequent intervals.

> *Note:* While the $T_3$ uptake test is a very useful aid in the evaluation of thyroid function, it should not be used as the sole basis for such an evaluation. In any patient, the clinical state is probably the best indication of thyroid status, and any laboratory test must be interpreted with caution when test results do not agree with clinical evidence.

The Thyrostat-3 test offers further advantages in the performance of the $T_3$ uptake test. Unlike many of the $T_3$ uptake procedures employing anion exchange resins, Thyrostat-3 test results are not significantly affected by variations in time or temperature during contact with the Thyrostat-3 Adsorbent Tablet (test results are essentially unchanged at normally encountered room temperatures ranging between 20° and 25° C.).

The use of $^{125}I$ rather than $^{131}I$ considerably lengthens the shelf-life of the liothyronine employed in the test because of the longer half-life of $^{125}I$ and the fact that it emits no beta rays to affect the stability of the liothyronine. Moreover, with $^{125}I$ labeled material, radiation exposure to the technician is lowered. Radioactivity is well within good counting range of modern equipment, and *in vitro* counting is quite efficient.

The half-life of $^{125}I$ is 60.2 days. The isotope decays in a complex fashion with emission of x-rays and gamma rays whose radiation energies are 27.4 keV and 35.4 keV, respectively. There is no beta emission.

## REAGENTS

The Thyrostat-3 *in vitro* diagnostic test for quantitative measurement of serum liothyronine ($T_3$) uptake for the evaluation of thyroid function is available in 25- and 100-test kits.

The Thyrostat-3, 25-test kit provides 25 plastic test tubes; 1 vial of Adsorbent Tablets (28 tablets); 1 bottle of Liothyronine I 125 Buffer Solution (50 ml, containing 0.03 μCi I 125 or less per ml with preservatives and buffer); and 1 Control Pak



J3-358B


100 ml

List G2105

# $^{125}$I T3 REAGENT

For use with T3 CLASP® RIA Kit
For *in vitro* Diagnostic Use • See directions
**Not for Internal or External Use in Humans or Animals**
**SODIUM AZIDE: 0.1%**
**Store at 2° to 8° C • SHAKE WELL BEFORE USE**

| | | |
|---|---|---|
| Total Act.: <4.3 microcuries | As of | Noon EST |
| LOT NO.: | EXP. DATE: | |

E. R. Squibb & Sons, Inc., Princeton, NJ 08540
Made in USA

C6303B / G2105


# T3 CLASP® RIA Kit

List G2105

Squibb T3 Radioimmunoassay Kit
For *in vitro* Diagnostic Use • See enclosed directions
**Not for Internal or External Use in Humans or Animals**
Contains sufficient material for 100 tubes
**Store at 2° to 8° C**

CONTENTS:
1 bottle (100 ml) $^{125}$I T3 REAGENT
<4.3 microcuries $^{125}$I per bottle
6 bottles (1.5 ml ea.) T3 RIA STANDARD
(1 each of 10, 50, 100, 250, 500 and 1000 ng per dl)

E. R. Squibb & Sons, Inc., Princeton, NJ 08540
Made in USA

M5371B / G2105

SQUIBB

J3-447B

Revised February 1980

# T3 CLASP® RIA Kit

## For Quantitative Measurement of Serum Liothyronine Levels by Radioimmunoassay

### For *IN VITRO* Diagnostic Use

### For Professional Use Only

**DETERMINATION OF SERUM LIOTHYRONINE ($T_3$) LEVELS BY RADIOIMMUNOASSAY**

Measurement of body constituents or administered compounds by the technique of radioimmunoassay offers a bioanalytical tool that combines the extreme sensitivity of radioisotope methodology with the extreme specificity of immunological techniques. The procedure requires a specific antibody, a radiolabeled antigen, a pure sample of the antigen to serve as a reference standard, and a means of separation of free antigen from antibody-bound antigen. The procedure follows the basic principle of saturation analysis, where there is competition between labeled and unlabeled antigen for a fixed number of antibody-binding sites. As the concentration of unlabeled antigen (the substance actually being measured) increases, less of the added radiolabeled antigen will be bound to the antibody. After a specified period of time, the bined with a normal serum thyroxine concentration is evidence for $T_3$ thyrotoxicosis, a disease state described by Sterling and co-workers in 1970.[1] Although liothyronine levels are usually below the normal range in hypothyroidism, some overlap with euthyroid levels has been found. Therefore, liothyronine assay results have limited use in the confirmation of hypothyroidism.

Earlier methods for the assay of serum liothyronine concentration (competitive protein binding[2,3] and gas chromatography[4]) were tedious, time consuming, and required large volumes of serum. The development of specific liothyronine antibodies[5] and the utilization of liothyronine-binding protein inhibitors[6,7] led to the development of specific, accurate, and relatively simple RIA procedures for the determination of serum liothyronine levels.

The utilization of solid-phase technology in the T3 CLASP

SQUIBB

CAUTION
RADIOACTIVE MATERIAL

Medotopes®

100 ml

List H0820

# T3 PREMIX

$^{125}$I T3 and Separant Mixture
For *in vitro* Diagnostic Use • See insert
Not for Internal or External Use in Humans or Animals
SODIUM AZIDE: 0.1% added
REFRIGERATE • SHAKE WELL BEFORE USE

Total Act.: < 6 microcuries $^{125}$I
As of Noon EST
LOT NO.:
EXP. DATE:

E. R. Squibb & Sons, Inc., Princeton, NJ 08540
Made in USA

C6747 / H0820

SQUIBB

CAUTION
RADIOACTIVE MATERIAL

Medotopes®

List H0820

# T3 SQUIBB RADIOIMMUNOASSAY KIT

For *in vitro* Diagnostic Use • **WARNING: NOT FOR INJECTION**
**Not for Internal or External Use in Humans or Animals**
Contains sufficient material for 100 tubes
**REFRIGERATE** • See insert

CONTENTS:
1 bottle (100 ml) T3 PREMIX <6 microcuries $^{125}$I per bottle
1 bottle (50 ml) T3 ANTISERUM
6 vials (1.2 ml ea.) T3 STANDARD (1 each of 0, 50, 100, 250, 500 and 1000 ng of triiodothyronine per dl)

T3 STANDARDS contain human serum

Restricted Device: Federal law restricts the sale, distribution, or use of this device to, by, or on the lawful order of, a health professional

E. R. Squibb & Sons, Inc., Princeton, NJ 08540
Made in USA

C5165 / H0820

# T3-SQUIBB RADIOIMMUNOASSAY Kit

For Quantitative Measurement of Serum
Triiodothyronine Levels by Radioimmunoassay

For *IN VITRO* Diagnostic Use

## FUNCTION OF THE TEST

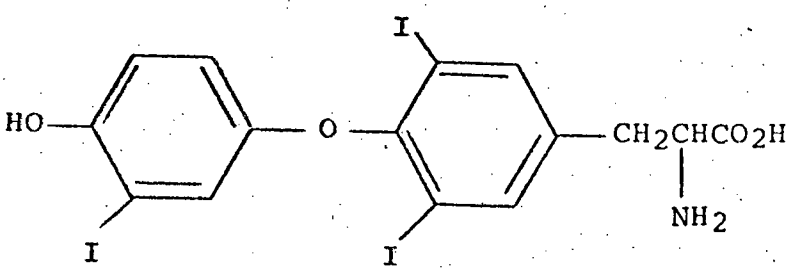
Measurement of serum levels of triiodothyronine ($T_3$) is an important adjunct in the determination of thyroid function. It is especially useful in the confirmation of hyperthyroidism and in the detection of $T_3$ thyrotoxicosis. An elevated serum triiodothyronine level accompanied by an elevated serum throxine level is a strong indication of hyperthyroidism. In patients exhibiting clinical hyperthyroidism, an elevated serum triiodothyronine level combined with a normal serum thyroxine concentration is evidence for $T_3$ thyrotoxicosis, a disease state described by Sterling and co-workers in 1970.[1] Disagreement exists concerning the applicability of triiodothyronine measurement to the detection of hypothyroidism. In all[2,3] or most[4] patients with unequivocal clinical hypothyroidism, levels of triiodothyronine are below normal. However, in borderline cases, triiodothyronine levels are sometimes[3] or often[2] normal. Thus, a reduced serum level of triiodothyronine is not as clear an indicator of hypothyroidism as a reduced serum level of thyroxine ($T_4$) or an elevated level of thyroid-stimulating hormone (TSH).[4]

## Chemical and Biological Principles

The basal membrane of the thyroid gland traps inorganic iodide and actively transports it into the gland where it is processed into organic iodide through conversion of the tyrosyl residues of the thyroglobulin to monoiodotyrosine and diiodotyrosine. Oxidative coupling of two diiodo residues yields $T_4$, whereas oxidative coupling of a monoiodo

residue to a diiodo residue yields $T_3$. Approximately a two month supply of $T_4$ is stored within the thyroglobulin. When needed, thyroglobulin is enzymatically hydrolyzed to release these hormones into the circulatory system. However, most of the $T_3$ in circulation is normally derived from peripheral deiodination of $T_4$ in the liver.

The release of $T_4$ and $T_3$ from the thyroid is markedly influenced by pituitary thyroid-stimulating hormone which in turn is influenced by hypothalamic thyrotropin-releasing hormone (TRH). Normally, increased blood levels of $T_4$ and $T_3$ act to decrease the amount of TSH secreted, thereby reducing the production and release of $T_4$ and $T_3$. Decreased blood levels of $T_4$ and $T_3$ produce the opposite effect, leading to increased production and secretion of $T_4$ and $T_3$. In this manner a normal circulating thyroid hormone balance is maintained. Most of the circulating $T_4$ and $T_3$ in the blood is bound to serum proteins, i.e., thyroxine-binding globulin (TBG), thyroxine-binding prealbumin (TBPA), and albumin. A small fraction of triiodothyronine (~0.3%) is free. This free triiodothyronine is considered to be the metabolically active form in its effect on target tissue. The free fraction of $T_3$ is influenced not only by the amount of thyroxine-binding proteins in the blood, but also by the rate of peripheral deiodination of $T_4$.




Triiodothyronine

PRINCIPLES OF THE TEST

The technique of radioimmunoassay offers a bioanalytical tool that combines the extreme sensitivity of radioisotope methodology with the extreme specificity of immunological techniques. The procedure requires a specific antibody, a radiolabeled antigen, a pure sample of the antigen to serve as a reference standard, and a means of separation of free antigen from antibody-bound antigen. The procedure follows the basic principle of saturation analysis, where there is competition between labeled and unlabeled antigen for a fixed number of antibody binding sites. As the concentration of unlabeled antigen (the substance actually being measured) increases, less of the radiolabeled antigen will be bound to the antibody. This competitive reaction is depicted in Figure 1. After a specified period of time, the free and bound components of the mixture are separated, and the radioactivity of the bound components is measured. The absolute quantity of unlabeled antigen in the sample being analyzed is determined by comparing the assay results to a standard curve prepared with known amounts of the unlabeled antigen.

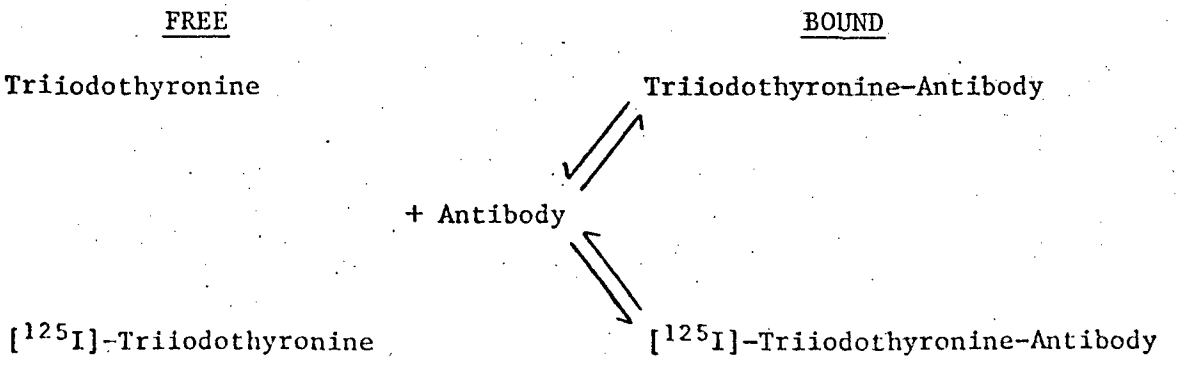



Figure 1. The competitive binding reaction.

In the T3-SQUIBB RADIOIMMUNOASSAY Kit, antibody to triiodothyronine (produced in New Zealand white rabbits by administration of a derivative of triiodothyronine that was coupled to a protein carrier) is the specific antibody, purified triiodothyronine containing $^{125}I$ serves as the radiolabeled antigen, and purified triiodothyronine is the reference standard. Specific components in the T3 Premix (see REAGENTS section) promote the release of triiodothyronine from the serum binding proteins. Separation of the free and bound radiolabeled antigen is achieved by a double antibody accelerator system consisting of goat anti-rabbit gamma-globulin and polyethylene glycol. In order to reduce the number of pipetting steps involved in the assay procedure, the radiolabel and the separant have been combined into a single reagent.

The half-life of $^{125}I$ is 60.2 days. The isotope decays in a complex fashion with emission of x-rays and gamma rays whose radiation energies are 27.4 keV and 35.4 keV, respectively. These energies are well within the detection capability of modern solid crystal gamma counters. There is no beta emmission.

Triiodothyronine levels are expressed in nanograms (ng, or $10^{-9}$ g) of triiodothyronine per deciliter of serum (ng/dl).

REAGENTS

The T3-SQUIBB RADIOIMMUNOASSAY Kit contains sufficient material for 100 tubes. Each kit contains the following components:

1. T3 Antiserum: 50 ml, including less than 5 microliters of $T_3$ antiserum per bottle, bovine serum albumin and normal rabbit serum (carriers), tris(hydroxymethyl)aminomethane and acetic acid (buffering agents), and sodium azide (a preservative) in water.

2. T3 Premix ($^{125}$I T3 and Separant Mixture): 100 ml, with a total radioactivity of less 6 microcuries of $^{125}$I per bottle; in an aqueous matrix containing goat anti-rabbit gamma-globulin (the second antibody); polyethylene glycol 4000 (the accelerator); disodium edetate dihydrate, sodium salicylate, and ammonium 8-anilino-1-naphthalene sulfonate (inhibitors of TBP binding); bovine serum albumin (a protein carrier); tris(hydroxymethyl)aminomethane and acetic acid (buffering agents); and sodium azide (a preservative).
3. T3 Standards: six vials, 1.2 ml each, containing 0, 50, 100, 250, 500, and 1,000 ng of triiodothyronine per dl in a matrix of human serum including sodium azide (a preservative) and trace amounts of ethanol and ammonium hydroxide (solubilizers).

THE T3 PREMIX MUST BE SHAKEN WELL IMMEDIATELY BEFORE USE.

Reagents within each kit are specific to the lot number on the kit; therefore, only reagents from kits with the same lot number may be used interchangeably.

All reagents and samples must be equilibrated to room temperature before use.

Warnings

For *IN VITRO* Diagnostic Use.

Restricted Device: Federal law restricts the sale, distribution, or use of this device to, by, or on the lawful order of, a health professional.

Note: This radioactive material may be received, acquired, possessed, and used only by physicians, veterinarians in the practice of veterinary medicine, clinical laboratories, or hospitals and only for in vitro clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use, and transfer are subject to the regulations and a general license of the US Nuclear Regulatory Commission or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority.

E.R. Squibb and Sons, Inc.

In vitro clinical laboratory testing with the T3-SQUIBB RADIOIMMUNOASSAY KIT requires only a general license from the US Nuclear Regulatory Commission. The general license is issued to any physician, clinical laboratory, or hospital obtaining a validated registered USNRC Form 483. This form must be submitted in triplicate to the USNRC. The possessor of a general license is subject to the conditions and limitations under 10 CFR 31.11. (A specific license is available from the USNRC for quantities larger than 200 microcuries.)

Precautions

Observe the following safety rules in handling radioactive material:

- There should be no pipetting by mouth, smoking, or consumption of any food or drink in areas where radioactive materials are permitted.
- Wear gloves when handling radioactive materials.
- Wash hands thoroughly after handling radioactive materials.
- Working areas should be covered with disposable absorbent paper.
- Wipe up spills quickly and thoroughly and discard the contaminated materials with the radioactive waste.
- Solid waste may be stored in a specifically designated area in a covered metal or plastic container that has been identified with a USNRC radiation caution label until the waste decays to a safe level. Then, after defacing the labeling, the material may be discarded with nonradioactive waste.
- The user should determine whether USNRC and/or local regulations permit the liquid radioactive waste to be discarded through the sanitary sewerage system.

Because T3 Standards contain human serum, these reagents, and patient samples, should be handled in the same manner as any potentially infective biological material. The human serum in the standards has been found to be nonreactive for $HB_sAg$ when tested with licensed reagents.

Chemical Hazard

- All of the reagents in this kit contain the commonly used bacteriostatic agent, sodium azide. Disposal of this material through the laboratory plumbing system could lead to the formation of highly explosive copper and lead azides. Therefore, it is recommended that large amounts of water be used to flush excess reagents through the plumbing system.

Storage

The kit should be refrigerated upon receipt.

All reagents should be stored in their original containers, with their original closures. Do not transfer these reagents to other containers or change the closures for storage purposes.

SPECIMEN COLLECTION AND PREPARATION

An appropriate quantity of blood should be collected from a peripheral vein using a standard blood collection tube that does not contain anticoagulant. The patient does not need to be in a fasting state when the blood is withdrawn.

Serum, not plasma, should be used in performing the test. The variables introduced into the procedure through the use of plasma could make the results unreliable.

It is preferable to separate the serum sample on the same day that the blood is withdrawn from the patient. Serum samples may be stored in

a refrigerator for up to 24 hours, and should be frozen if stored for longer periods. Frozen samples should be equilibrated to room temperature and mixed well before use. Samples should not be refrozen.

The use of hemolyzed or lipemic samples is not recommended.

The patient's history should be thoroughly scrutinized to determine if any diagnostic or therapeutic radioisotopes have been administered within the week or two immediately prior to the $T_3$ determination. If the patient has been treated with radioisotopes or if the patient's history is unavailable, the activity of the serum sample should be checked in a scintillation well counter that is set for $^{125}I$. If after a few seconds it appears that the count rate is above background, 20 microliters of the serum sample should be accurately counted to determine whether the radioactivity contained in this volume could significantly affect assay results.

TEST PROCEDURE

Materials Needed

In addition to the reagents supplied with the T3-SQUIBB RIA Kit, the following materials are required:

- 12 X 75 mm polystyrene test tubes
- Centrifuge capable of 3000 to 4000 rpm
- Well-type gamma scintillation counter (discriminator settings of 20-50 keV)
- Refrigerator
- Vortex mixer
- Pipettor and tips for 100 microliters (µl) [and, optionally 500 µl]
- Repeating pipettors capable of delivering 1 ml and 500 µl
- Container for radioactive waste
- Test tube racks

Precedural Precautions

Good laboratory practice dictates that an appropriate number of controls be analyzed in conjunction with each set of samples.

All reagents and samples must be equilibrated to room temperature before use.

Procedure

The technique described below is based upon the performance of duplicate analyses for all standards, samples and controls. Read the entire procedure before starting the test.

1. Mark a series of 12 X 75 mm plastic test tubes with numerals 1 through 14. The first two tubes are used for measuring total counts and the remaining 12 tubes are required for generation of a standard curve. Two additional tubes will be needed for each clinical sample or control to be assayed.*

2. Add 100 µl of T3 Standard or 100 µl of the clinical sample to the respective tubes as follows:

| Tubes | Sample to be Added |
|---|---|
| 1 and 2 | None |
| 3 and 4 | 100 µl of 0 ng/dl Standard |
| 5 and 6 | 100 µl of 50 ng/dl Standard |
| 7 and 8 | 100 µl of 100 ng/dl Standard |
| 9 and 10 | 100 µl of 250 ng/dl Standard |
| 11 and 12 | 100 µl of 500 ng/dl Standard |
| 13 and 14 | 100 µl of 1000 ng/dl Standard |
| 15 and 16 | 100 µl of Clinical Sample |

3. Add 1.0 ml of T3 Premix to each tube. THIS REAGENT MUST BE SHAKEN WELL IMMEDIATELY BEFORE USE.

4. Add 500 µl of T3 Antiserum to tubes 3 through 14.

5. Vortex each tube.

6. Set tubes 1 and 2 aside and incubate the remaining tubes at ambient temperature for 2 hours.

*If desired, nonspecific binding can be determined as follows:

a) Mark two tubes as NSB.
b) Add 100 ml of "0" Standard to each tube.
c) Add 500 ml of distilled water to each tube.
d) Add 1.0 ml of T3 Premix to each tube.
e) Treat these two tubes in the same manner as tubes 3 through 14 by completing steps 5 through 10.

7. Centrifuge the incubated tubes for 5 to 10 minutes at 3000 to 4000 rpm.

8. Decant the supernatant by gently inverting each tube once, discarding the liquid into a radioactive waste container. Keeping the tube inverted, touch the rim on an absorbent paper and gently tap the tube.

9. Measure the radioactivity in all tubes in a standard well-type gamma scintillation counter (discriminator settings of 20 to 50 keV) for one minute or for a fixed amount of time that is sufficient to eliminate counting statistics as an important source of variability.

10. Subtract background cpm and record net cpm for each tube.

RESULTS

1. It is possible to plot either % Bound or B/Bo or net cpm versus concentration on semilog graph paper. Use the following formula to calculate % Bound for each tube and record the results on a worksheet.

$$\% \text{ Bound} = \frac{\text{PELLET COUNTS (net cpm)} \times 100}{\text{TOTAL COUNTS (net cpm)}}$$

Example:

Background cpm: 150
Tube #3 PELLET COUNTS (gross cpm): 52,398
TOTAL COUNTS (gross cpm): 98,227

$$\% \text{ Bound} = \frac{52{,}398 - 150}{98{,}227 - 150} \times 100$$

$$= \frac{52{,}248}{98{,}077} \times 100 = 53.3$$

Alternately, use the following formula to calculate B/Bo values for each tube and record the results on a worksheet.

$$B/Bo = \frac{\text{PELLET COUNTS (net cpm)}}{\text{Avg. PELLET COUNTS OF "0" Std. (net cpm)}}$$

Example:

Background cpm: 150
Patient #1 PELLET COUNTS (gross cpm): 25,150
"0" Std. - Avg. PELLET COUNTS (gross cpm): 50,150

$$B/Bo = \frac{25,150 - 150}{50,150 - 150}$$

$$= \frac{25,000}{50,000} = 0.50$$

2. Prepare a standard curve by plotting either % Bound or B/Bo or net cpm against the concentrations of the standards. A typical set of data calculated in terms of % Bound is given in Table I and graphed in Figure II. The same set of data calculated in terms of B/Bo is listed in Table II and plotted in Figure III. These curves are provided for guidance only and should not be used in calculating the $T_3$ levels in the clinical samples.

3. Determine the concentration (ng/dl) of $T_3$ in each sample by referring to the standard curve prepared earlier. The observed % Bound or B/Bo or net cpm for each sample will correspond to a specific $T_3$ concentration. Samples with $T_3$ concentrations of greater then 1000 ng/dl should be accurately diluted with "0" standard and reassayed, if an exact value is required.

TABLE I

Typical Standard Curve Data
Calculated in Terms of % Bound

| TOTAL COUNT | | | 69,700 cpm |
|---|---|---|---|
| Standard Concentration (ng/dl) | Pellet Counts (net cpm) | % Bound | Avg. % Bound |
| 0 | 38,551 | 55.3 | |
| 0 | 38,606 | 55.4 | 55.4 |
| 50 | 31,081 | 44.6 | |
| 50 | 31,652 | 45.4 | 45.0 |
| 100 | 26,712 | 38.3 | |
| 100 | 26,706 | 38.3 | 38.3 |
| 250 | 17,841 | 25.6 | |
| 250 | 17,860 | 25.6 | 25.6 |
| 500 | 11,522 | 16.5 | |
| 500 | 11,625 | 16.7 | 16.6 |
| 1000 | 7,274 | 10.4 | |
| 1000 | 7,189 | 10.3 | 10.4 |

TABLE II

Typical Standard Curve Data
Calculated in Terms of B/Bo

| TOTAL COUNT | | | 69,700 cpm |
|---|---|---|---|
| Bo Counts (net avg.) | | | 38,578 cpm |
| Standard Concentration (ng/dl) | Pellet Counts (net cpm) | B/Bo | Avg. B/Bo |
| 0 | 38,551 | 1.00 | |
| 0 | 38,606 | 1.00 | 1.00 |
| 50 | 31,081 | 0.80 | |
| 50 | 31,652 | 0.82 | 0.81 |
| 100 | 26,712 | 0.69 | |
| 100 | 26,706 | 0.69 | 0.69 |
| 250 | 17,841 | 0.46 | |
| 250 | 17,860 | 0.46 | 0.46 |
| 500 | 11,522 | 0.30 | |
| 500 | 11,625 | 0.30 | 0.30 |
| 1000 | 7,274 | 0.19 | |
| 1000 | 7,189 | 0.19 | 0.19 |

LIMITATIONS OF THE TEST PROCEDURE

In an individual having normal levels of TBG, the measurement of total $T_3$ yields an accurate diagnosis of thyroid status. However, there are many circumstances in which the level of TBG is not normal. For example, pregnancy or estrogen therapy cause increased synthesis of TBG and a concomittant increase in total $T_3$ whereas androgenic steroids have the opposite effect. Because of this variation in TBG levels, interpretation of $T_3$ results should be tempered by the determination of TBG binding capacity via the $T_3$ uptake assay.*

In addition, depressed levels of triiodothyronine have been observed in a wide variety of serious, nonthyroidal illnesses such as hepatic cirrhosis, anorexia nervosa, chronic renal failure, and disseminated malignancy,[5-7] after surgery,[8] and during caloric restriction.[5]

EXPECTED VALUES

Serum samples from 110 normal volunteers were assayed with the T3-SQUIBB RADIOIMMUNOASSAY Kit obtaining the following results:

| | |
|---|---|
| Arithmetic Mean | 115.3 ng/dl |
| Standard Deviation | 17.5 ng/dl |
| Observed Range | 70 - 155 ng/dl |

Each sample was analyzed in duplicate. The frequency distribution of the patient values is depicted in Figure IV. With these results, the percentile estimate method yielded a normal range of 86 to 151 ng/dl, with 95% confidence.

It is recommended that each laboratory establish its own range of normal triiodothyronine values.

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*$T_3$ uptake may be determined by use of the Squibb THYROSTAT®-3 Diagnostic Test Kit.

In another study 46 patient samples were analyzed by the T3-SQUIBB RADIOIMMUNOASSAY Kit, the T3RIA (PEG) Diagnostic Kit of Abbott Laboratories, and the IMMOPHASE™ T-3 $^{125}I$ Radioimmunoassay Test System of Corning Medical. The regression analyses of these data are shown in Figures V and VI.

SPECIFIC PERFORMANCE CHARACTERISTICS

Standard Curve

Typical standard curves are shown in Figures II and III.

Sensitivity

The lowest triiodothyronine-containing standard supplied with the kit contains 50 ng/dl.

Specificity

The cross-reactivity of the antiserum with various substances is tabulated in Table III.

TABLE III

Cross-Reactivity of Various Substances with the T3-SQUIBB RADIOIMMUNOASSAY Kit

| Substance | Concentration | Percent Cross-Reactivity |
|---|---|---|
| 3,5-Diiodothyronine ($T_2$) | 50 µg/dl | 0.5 |
| Diphenylhydantoin | 10 mg/dl | 0 |
| Iodoacetic acid | 50 µg/dl | 0 |
| Phenyl butazone | 10 mg/dl | 0 |
| Sodium salicylate | 50 mg/dl | 0 |
| Reverse Triiodothyronine ($rT_3$) | 50 µg/dl | 0.04 |
| L-Thyroxine ($T_4$) | 40 µg/dl | 0.05 |
| | 23 µg/dl | 0 |

Recovery Efficiency

An important criterion of assay validity is the recovery of exogenous triiodothyronine from serum samples. Various amounts of exogenous $T_3$ were added to a serum pool having an endogenous $T_3$ content of 51 ng/dl and each sample was then assayed in duplicate. The results, which are presented in Table IV, demonstrate quantitative recovery of the exogenous triiodothyronine.

TABLE IV

Recovery Efficiency

| Endogenous Concentration (ng/dl) | Amount Added (ng/dl) | Total Expected (ng/dl) | Total Found (ng/dl) | Percent Recovery* |
|---|---|---|---|---|
| 51 | 50 | 101 | 107 | 105.9 |
| 51 | 100 | 151 | 161 | 106.6 |
| 51 | 250 | 301 | 325 | 108.0 |
| 51 | 500 | 551 | 590 | 107.1 |

*Percent Recovery = $\frac{\text{Total Found}}{\text{Total Expected}} \times 100$

Parallelism

This type of study indicates whether patient samples and standards behave similarly toward the radiolabel and the antiserum, thereby validating the matrix of the standards and the use of the zero standard as a diluent for patient samples. A serum sample having a known analyte concentration is serially diluted with zero standard and the original sample and its dilutions are assayed. A plot of observed dose against expected dose should theoretically yield a 45° line through the origin. Such a study was performed with three separate samples containing high levels of triiodothyronine. The results, depicted in Figure VII, demonstrate the validity of the matrix for the standards and the use of zero standard as a diluent.

Precision

Intra-assay CV data were obtained by performing a single run that consisted of 20 consecutive tubes each of human serum pools containing low, medium and high concentrations of thyroxine. In order to obtain inter-assay CV data, each of these controls was also assayed in duplicate in 25 runs done over a period of two months. The observed results yielded the reproducibility data shown in Table V.

TABLE V

Precision Data

| Parameter | Serum Pool Low | Medium | High |
|---|---|---|---|
| INTRA-ASSAY | | | |
| Mean (ng/dl) | 79 | 158 | 313 |
| Std. Dev. (ng/dl) | 3.6 | 5.2 | 6.2 |
| Range (ng/dl) | 74-84 | 152-170 | 308-330 |
| Coefficient of Variation (%) | 4.6 | 3.3 | 2.0 |
| INTER-ASSAY | | | |
| Mean (ng/dl) | 74 | 156 | 320 |
| Std. Dev. (ng/dl) | 4.6 | 7.0 | 8.5 |
| Range (ng/dl) | 64-82 | 145-170 | 305-340 |
| Coefficient of Variation (%) | 6.2 | 4.5 | 2.7 |

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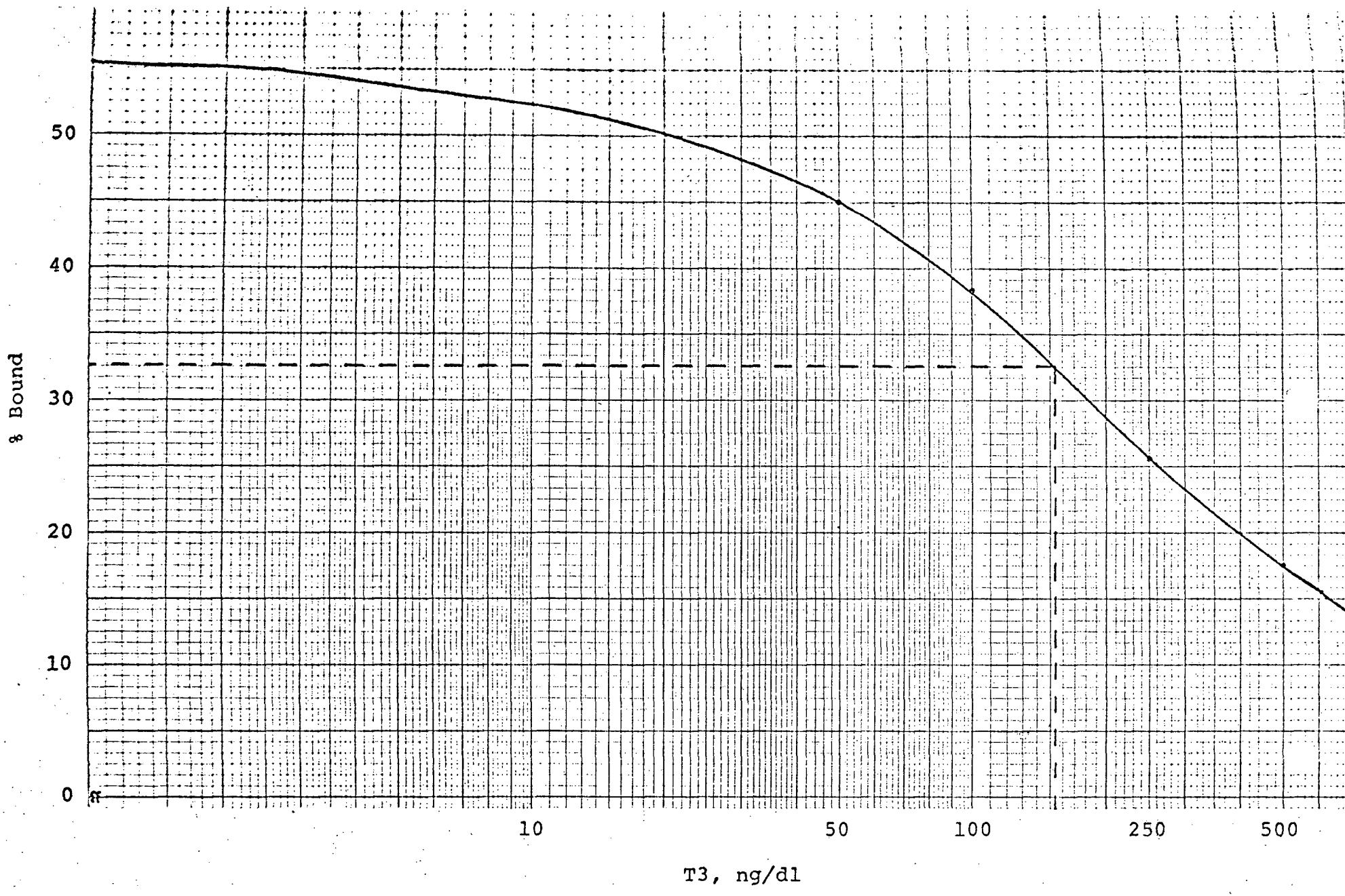



Figure II. A graph of % Bound vs. T3 Concentration for a typical standard curve obtained with the T3-SQUIBB RADIOIMMUNOASSAY KIT. As indicated, a patient sample having a mean % Bound of 32.5 would have a triiodothyronine concentration of 154 ng/dl.

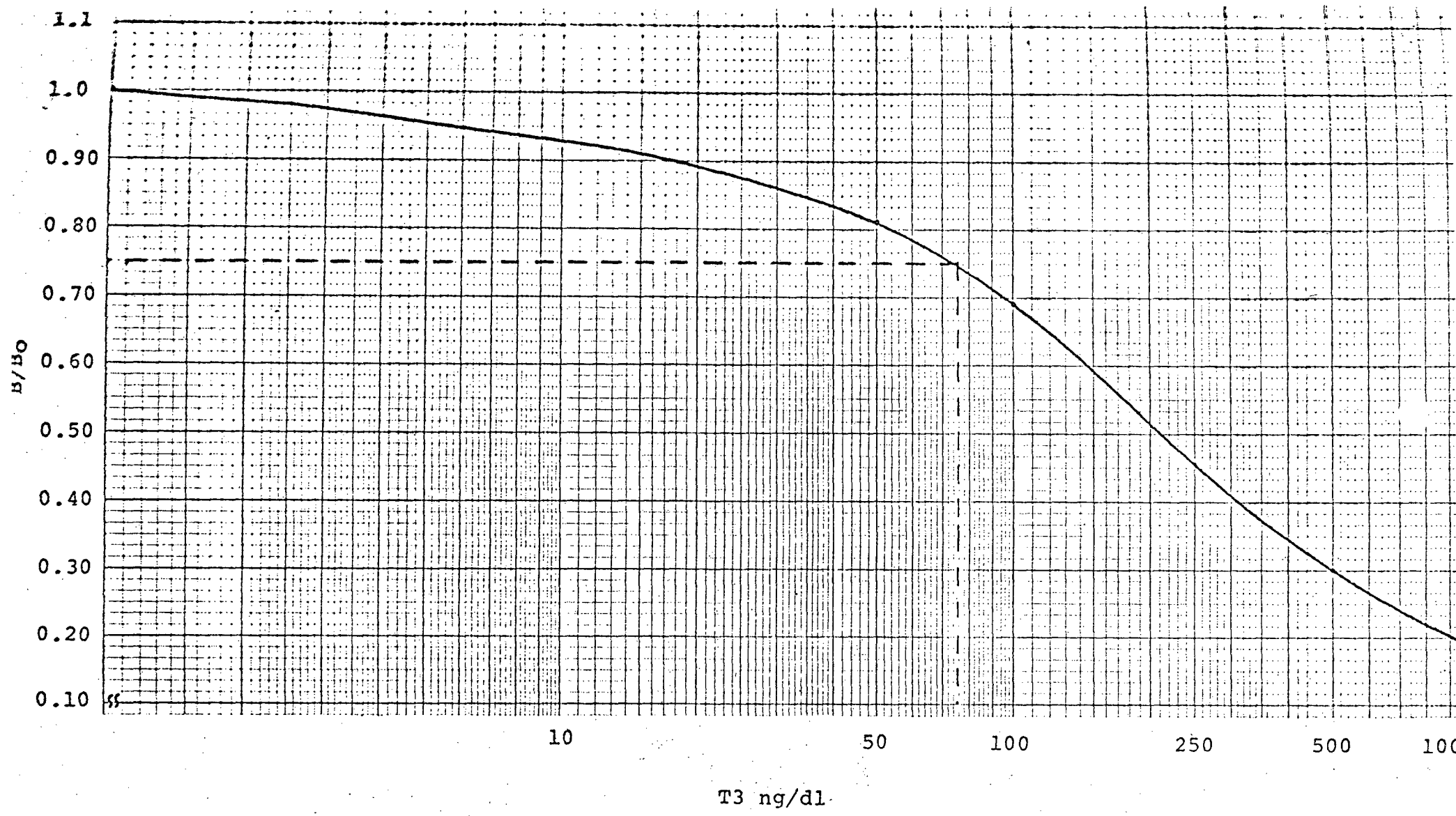



Figure III. A graph of $B/B_o$ vs. T3 Concentration for a typical standard curve obtained with the T3-SQUIBB RADIOIMMUNOASSAY KIT. As indicated, a patient sample having a mean $B/B_o$ of 0.75 would have a triiodothyronine concentration of 75 ng/dl.

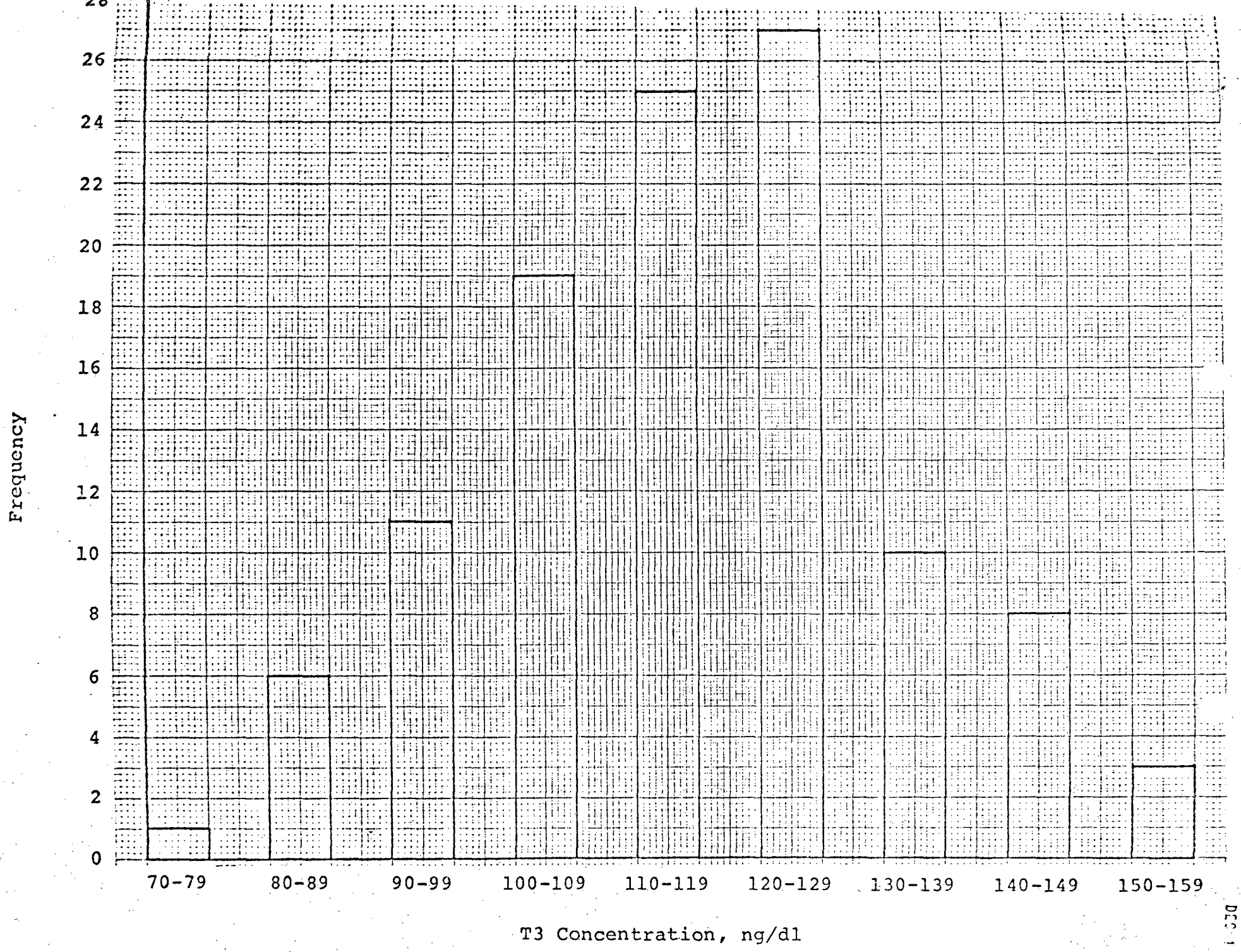



Figure IV. Frequency distribution of 110 samples from normal volunteers.

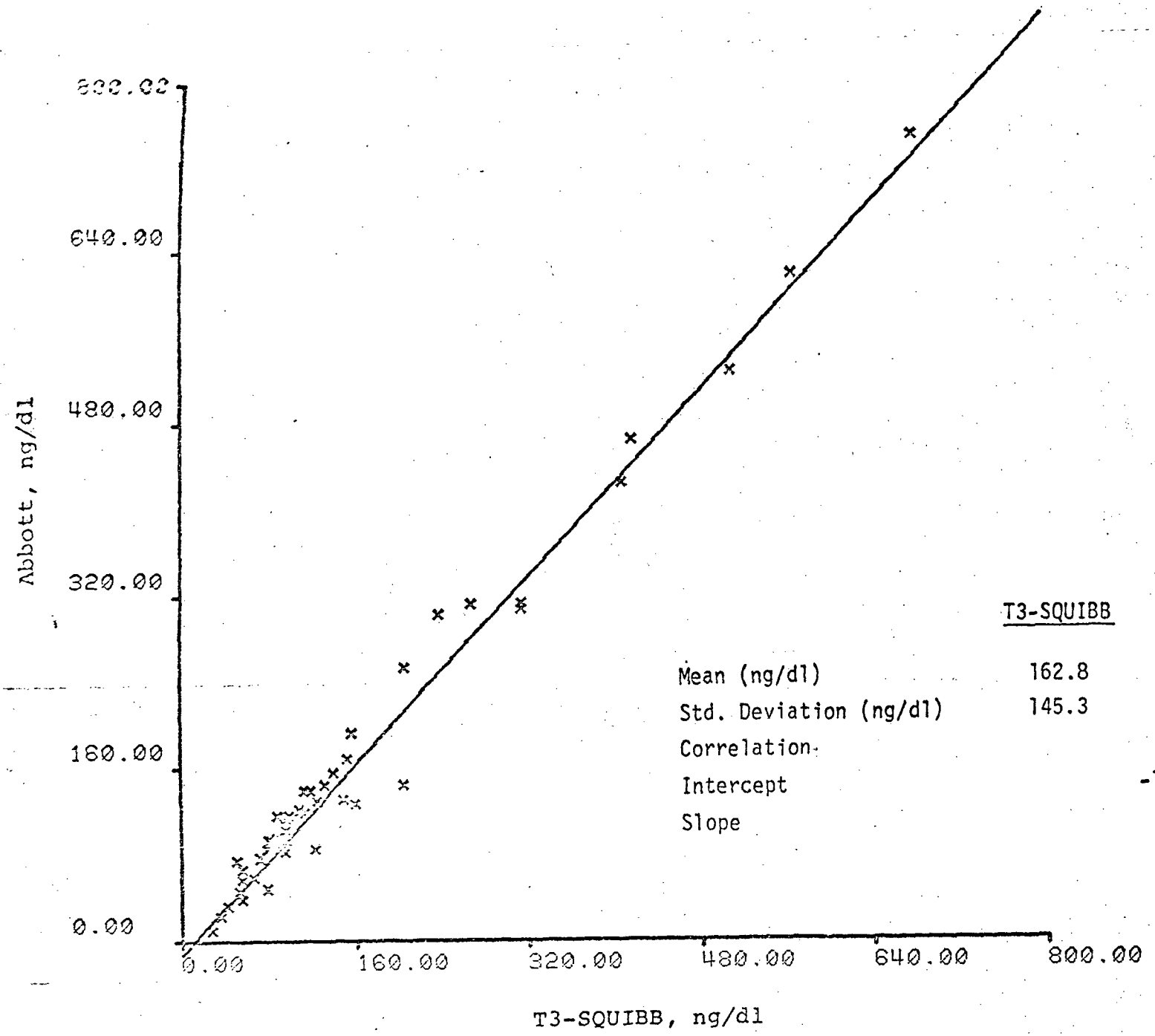



Figure V. Regression analysis of 46 samples analyzed by the T3-SQUIBB Radioimmunoassay Kit and the Abbott Laboratories T3RIA (PEG) Diagnostic Kit.

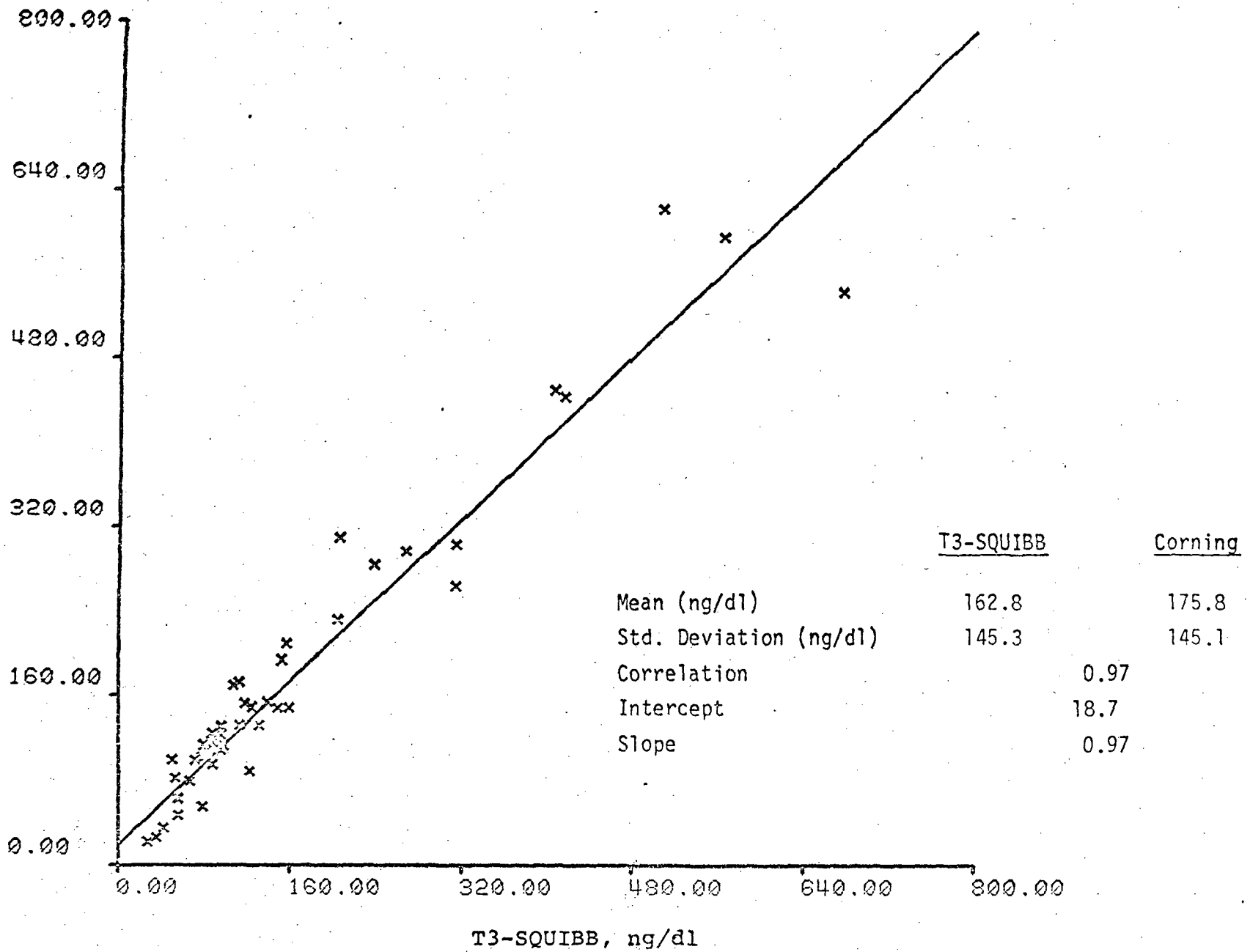



Figure VI. Regression analysis of 46 samples analyzed by the T3-SQUIBB Radioimmunoassay Kit and the Corning Medical IMMOPHASE™ T-3 $^{125}$I Radioimmunoassay Test System.

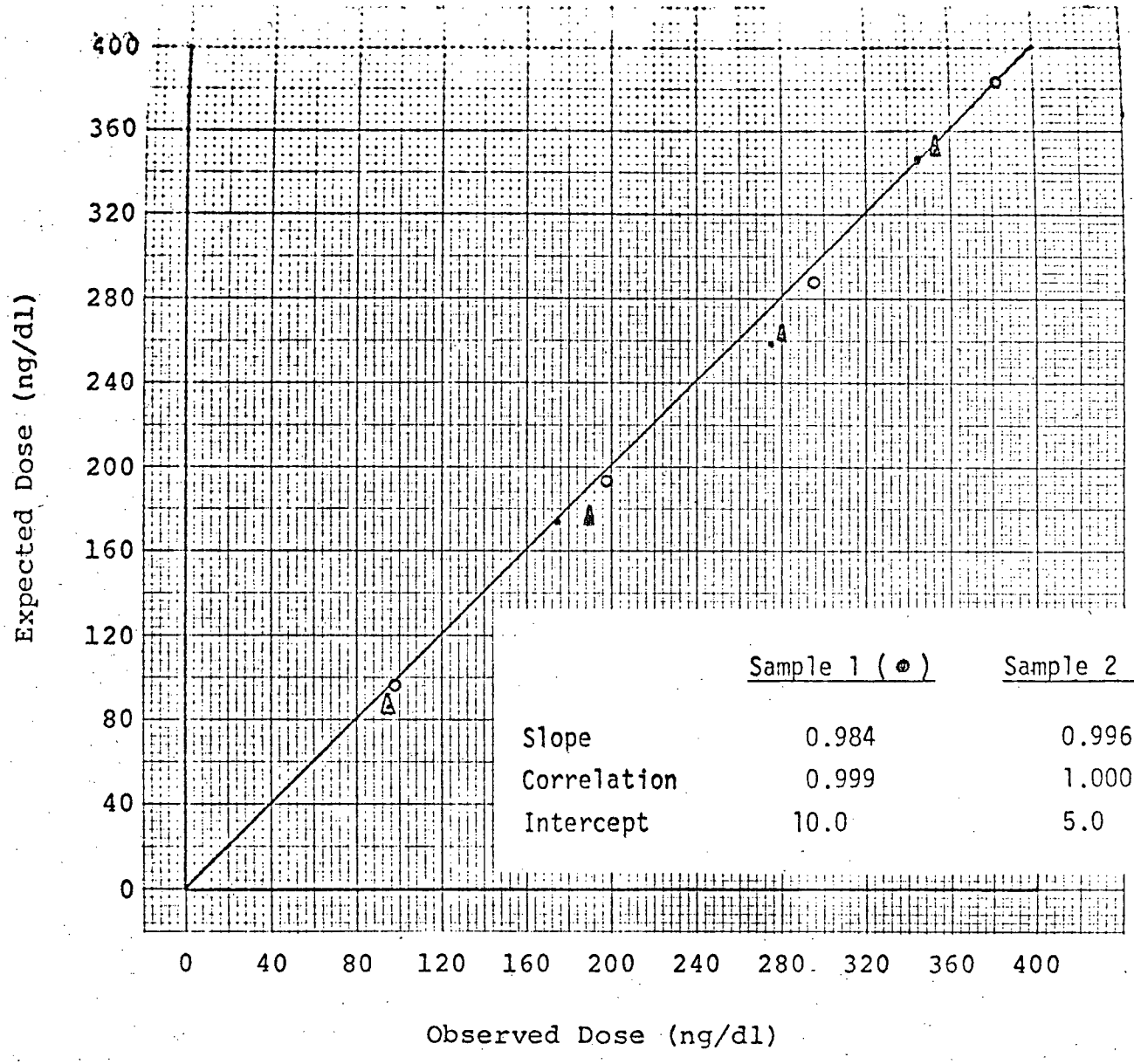



Figure VII. The parallelism study: A graph of observed dose versus expected dose. The theoretical 45° line is shown.

SQUIBB Store at 2° to 8° C CAUTION RADIOACTIVE MATERIAL

100 ml List G2110

# $^{125}$I T4 REAGENT

For use with T4 CLASP® RIA Kit
For *in vitro* Diagnostic Use • See directions
Not for Internal or External Use in Humans or Animals
SODIUM AZIDE: 0.1%
SHAKE WELL BEFORE USE

| | | |
|---|---|---|
| Total Act.: <3.96 microcuries $^{125}$I | As of | Noon EST |
| LOT NO.: | EXP. DATE: | |

E. R. Squibb & Sons, Inc., Princeton, NJ 08540
Made in USA C6286A / G2110

SQUIBB CAUTION RADIOACTIVE MATERIAL Radiocaps

# T4 CLASP® RIA Kit

List G2110

Squibb T4 Radioimmunoassay Kit
For *in vitro* Diagnostic Use • See enclosed directions
**Not for Internal or External Use in Humans or Animals**
Contains sufficient material for 100 tubes
**Store at 2° to 8° C**

CONTENTS:
1 bottle (100 ml) $^{125}$I T4 REAGENT
<3.96 microcuries $^{125}$I per bottle
5 bottles (1 ml ea.) T4 RIA STANDARD
(1 each of 1, 5, 10, 15 and 25 µg per dl)
1 bottle (1 ml) T4 RIA CONTROL

E. R. Squibb & Sons, Inc., Princeton, NJ 08540
Made in USA M5367A / G2110

06242

SQUIBB

J3-438A

Revised February 1980

# T4 CLASP® RIA Kit

## For Quantitative Measurement of Serum Thyroxine Levels by Radioimmunoassay

### For *IN VITRO* Diagnostic Use

### For Professional Use Only

**DETERMINATION OF SERUM THYROXINE LEVELS BY RADIOIMMUNOASSAY**

Measurement of body constituents or administered compounds by the technique of radioimmunoassay offers a bioanalytical tool that combines the extreme sensitivity of radioisotope methodology with the extreme specificity of immunological techniques. The procedure requires a specific antibody, a radiolabeled antigen, a pure sample of the antigen to serve as a reference standard, and a means of separation of free antigen from antibody-bound antigen. The procedure follows the basic principle of saturation analysis, where there is competition between labeled and unlabeled antigen for a fixed number of antibody binding sites. As the concentration of unlabeled antigen (the substance actually being measured) increases, less of the added radiolabeled antigen will be bound to the antibody. After a specific period of time, the other confirmatory tests such as TSH assay, serum liothyronine ($T_3$) uptake by the competitive protein-binding technique (CPB), $T_3$ by RIA, free thyroxine, or free thyroxine index (FTI) may be helpful in determining thyroid status.

Until recently the most commonly used method for the determination of total serum thyroxine utilized the thyroxine-binding properties of serum TBG in the CPB technique. The use of the more recently introduced radioimmunoassay technique for serum thyroxine determination offers several advantages over the CPB technique. Extraction of the thyroxine from serum is eliminated, thereby eliminating the problem of variation in extraction efficiency. The serum sample size required for RIA (10 µl) is much less than that used in the CPB technique; cases in which limited quantities of serum are available (e.g., pediatric or elderly patients), are more readily accommodated. Use of high-affinity antiserum in the RIA


50 ml **REFRIGERATE AT 2° to 8° C** List 09127

## THYROSTAT®-FTI BUFFER SOLUTION

Contains T4 Binding Globulin and I 125-Thyroxine
FOR LABORATORY USE ONLY with Thyrostat-FTI
Adsorbent Tablets • See accompanying directions
For *in vitro* Diagnostic Use • Shake well before using
SODIUM AZIDE: 0.1%
Not for Internal or External Use in Humans or Animals

Total Act. <2.6 microcuries I 125 As of Noon EST
LOT NO. EXP. DATE

E. R. Squibb & Sons, Inc.
Princeton, NJ 08540 Made in USA C6031B / 09127


25 TEST PKG.

## THYROSTAT®-FTI DIAGNOSTIC TEST KIT

For *in vitro* Diagnostic Use
For Evaluation of Thyroid Function
FOR LABORATORY USE ONLY
**Not for Internal or External Use in Humans or Animals**
**REFRIGERATE AT 2° to 8°C**

CONTENTS: List 09127
1 vial (28 tabs.) THYROSTAT-FTI ADSORBENT TABLETS
1 bottle (50 ml) THYROSTAT-FTI BUFFER SOLUTION
<2.6 microcuries I 125 per bottle
1 vial (3.0 ml) THYROSTAT-FTI CONTROL SERUM
1 vial (25 ml) THYROSTAT-FTI EXTRACTION ALCOHOL

NOTE: All reagents in this kit must be used with the accompanying Adsorbent Tablets • See accompanying directions

E. R. Squibb & Sons, Inc.
Princeton, NJ 08540 Made in USA C6040A / 09127

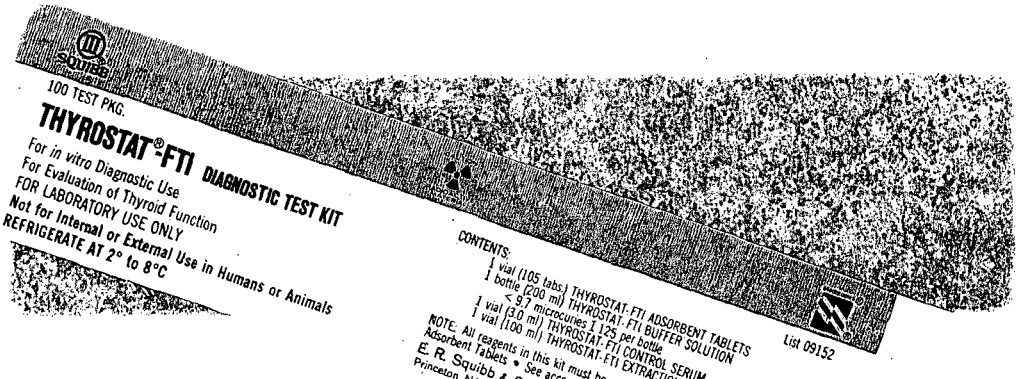

200 ml **REFRIGERATE AT 2° to 8° C** List 09152

## THYROSTAT®-FTI BUFFER SOLUTION

Contains T4 Binding Globulin and I 125-Thyroxine
FOR LABORATORY USE ONLY with Thyrostat-FTI
Adsorbent Tablets • See accompanying directions
For *in vitro* Diagnostic Use • **Shake well before using**
**SODIUM AZIDE: 0.1%**
**Not for Internal or External Use in Humans or Animals**

Total Act. <9.7 microcuries I 125 As of Noon EST
LOT NO. EXP. DATE

**E. R. Squibb & Sons, Inc.**
Princeton, NJ 08540 Made in USA C6034B / 09152




100 TEST PKG.

## THYROSTAT®-FTI DIAGNOSTIC TEST KIT

For *in vitro* Diagnostic Use
For Evaluation of Thyroid Function
FOR LABORATORY USE ONLY
**Not for Internal or External Use in Humans or Animals**
**REFRIGERATE AT 2° to 8°C**

CONTENTS: List 09152
1 vial (105 tabs.) THYROSTAT-FTI ADSORBENT TABLETS
1 bottle (200 ml) THYROSTAT-FTI BUFFER SOLUTION
<9.7 microcuries I 125 per bottle
1 vial (3.0 ml) THYROSTAT-FTI CONTROL SERUM
1 vial (100 ml) THYROSTAT-FTI EXTRACTION ALCOHOL

NOTE: All reagents in this kit must be used with the accompanying Adsorbent Tablets • See accompanying directions

E. R. Squibb & Sons, Inc.
Princeton, NJ 08540 Made in USA C6041A / 09152

SQUIBB®

CAUTION
RADIOACTIVE MATERIAL

Medotopes®

500 TEST KIT List 09172

# THYROSTAT®-FTI DIAGNOSTIC TEST KIT

For *in vitro* Diagnostic Use
For Evaluation of Thyroid Function
FOR LABORATORY USE ONLY• See accompanying directions
**Not for Internal or External Use in Humans or Animals**

CONTENTS:

500 plastic test tubes
5 vials (105 tabs. each) THYROSTAT-FTI ADSORBENT TABLETS
5 bottles (200 ml each) THYROSTAT-FTI BUFFER SOLUTION
<9.7 microcuries I 125 per bottle
5 vials (3.0 ml each) THYROSTAT-FTI CONTROL SERUM
5 vials (100 ml each) THYROSTAT-FTI EXTRACTION ALCOHOL

**Note:** All reagents in this kit must be used with the accompanying Adsorbent Tablets

**REFRIGERATE AT 2° to 8° C**

E. R. Squibb & Sons, Inc.
Princeton, NJ 08540 Made in USA C8041A / 09172

SQUIBB

J3-415C

# THYROSTAT®-FTI DIAGNOSTIC TEST KIT

Revised February 1980

## For Quantitative Measurement of Free Thyroxine Index for the Evaluation of Thyroid Function

## For *In Vitro* Diagnostic Use

## For Professional Use Only

### PRINCIPLES OF THE TEST

In the absence of abnormalities in serum thyroxine-binding proteins (TBP) concentration, the measurement of total serum thyroxine provides an accurate means of assessing thyroid status. In conditions where TBP concentration is altered, it is also necessary to obtain an estimate of the unoccupied binding sites of the TBP, in order to obtain an accurate indication of thyroid status. The Thyrostat-FTI procedure provides a means for simultaneous measurement of total serum thyroxine and estimation of unoccupied binding sites of the TBP. The result obtained by this procedure provides an indirect measure of free thyroid hormone, which correlates closely with thyroid status, regardless of serum TBP concentration.

The first step in the assay procedure involves liberation of the bound thyroxine and extraction of the major portion of the liberated and free thyroxine with an alcoholic solvent. Subsequent to the extraction step, the procedure follows the basic principle of saturation analysis and requires specific thyroxine-binding proteins, a radiolabeled derivative of thyroxine, and a normal control serum. With the saturation analysis technique, there is competition between labeled and unlabeled thyroxine for a fixed number of protein binding sites. As the concentration of unlabeled thyroxine (obtained from the extraction step) increases, less of the radiolabeled thyroxine will be retained by the binding proteins. The quantity of thyroxine which is not bound by the specific binding proteins is measured by introducing an adsorbent material that will only bind free thyroxine. The relative amounts of free and bound thyroxine are determined by isolating the adsorbent and measuring the radioactivity associated with it. The estimation of unoccupied binding sites of the TBP is achieved by adding a small quantity of unextracted patient serum to the incubation medium prior to addition of the adsorbent material. The amount of radiolabeled thyroxine taken up by the adsorbent material will be influenced by the number of unoccupied TBP binding sites present in the patient serum sample. As the number of unoccupied binding sites increases, the amount of radioactivity taken up by the adsorbent material will decrease. The net effect of addition of unextracted patient serum is adjustment of assay results to compensate for alterations in TBP concentration. Serum thyroxine measurements adjusted for TBP concentration provide an indirect measure of serum-free thyroxine, which is highly correlated with thyroid status. An "index" of free thyroid hormone is obtained by comparing adsorbent uptake of radiolabeled thyroxine in a patient sample with adsorbent uptake of radiolabeled thyroxine for a normal control serum sample assayed in an identical manner. When the thyroid gland produces an excess of thyroid hormones, as in hyperthyroidism, the increased level of free thyroxine will be reflected by an increased uptake of radiolabeled thyroxine by the adsorbent material, when compared with the uptake for a normal control serum. Conversely, when thyroid hormone production is decreased, as in hypothyroidism, the decreased level of free thyroxine will be reflected by a decreased adsorbent uptake of radiolabeled thyroxine, compared with the uptake for a normal control serum.

In the Thyrostat-FTI test, the thyroxine is extracted from serum with ethanol, and the alcoholic extract is mixed with $^{125}$I-labeled thyroxine bound to thyroxine-binding proteins. An organic adsorbent is used to separate bound thyroxine from free thyroxine. The normal control serum is of animal origin.

### NOMENCLATURE

It has been suggested by an *ad hoc* committee of the American Thyroid Association[1] that the measurement of total serum thyroxine by the classic Murphy-Pattee "radiotransin assay" be identified as "Thyroxine (displacement)," abbreviated "$T_4$(D)," and expressed as micrograms per 100 milliliters of serum (μg/100 ml).

When there are pronounced alterations in the binding capacity of the transport proteins, the $T_4$(D) test, used in conjunction with the commonly used $T_3$ test (Resin Triiodothyronine uptake-$RT_3U$), provides an indirect measure of the concentration of free $T_4$, which is highly correlated with the thyroid state.[2] This indirect measure of free $T_4$ is commonly referred to as the "free thyroxine index," with the American Thyroid Association suggested designation being "Thyroxine-resin $T_3$ index," abbreviated $T_4$-$RT_3$. The $T_4$-$RT_3$ is the mathematical product of the results of the $T_4$(D) and $RT_3U$ tests.

Nomenclature has not been suggested for tests that provide a direct measurement of "free thyroxine index," as opposed to the calculation procedure described above. For purposes of simplicity, the direct measurement of "free-thyroxine index," as embodied in the Thyrostat-FTI procedure, will be abbreviated FTI(M).

### RATIONALE FOR USE

The measurement of total serum thyroxine ($T_4$) by the $T_4$(D) procedure represents a significant advance in the *in vitro*