NRC FORM 313
(9-85)
10 CFR 30, 32, 33, 34, 35 and 40

U.S. NUCLEAR REGULATORY COMMISSION
APPROVED BY OMB
3150-0120
Expires: 5-31-87

APPLICATION FOR MATERIAL LICENSE

INSTRUCTIONS: SEE THE APPROPRIATE LICENSE APPLICATION GUIDE FOR DETAILED INSTRUCTIONS FOR COMPLETING APPLICATION. SEND TWO COPIES OF THE ENTIRE COMPLETED APPLICATION TO THE NRC OFFICE SPECIFIED BELOW.

FEDERAL AGENCIES FILE APPLICATIONS WITH:

U.S. NUCLEAR REGULATORY COMMISSION
DIVISION OF FUEL CYCLE AND MATERIAL SAFETY, NMSS
WASHINGTON, DC 20555

ALL OTHER PERSONS FILE APPLICATIONS AS FOLLOWS, IF YOU ARE LOCATED IN:

CONNECTICUT, DELAWARE, DISTRICT OF COLUMBIA, MAINE, MARYLAND, MASSACHUSETTS, NEW HAMPSHIRE, NEW JERSEY, NEW YORK, PENNEYLVANIA, RHODE ISLAND, OR VERMONT, SEND APPLICATIONS TO:

U.S. NUCLEAR REGULATORY COMMISSION, REGION I
NUCLEAR MATERIAL SECTION B
631 PARK AVENUE
KING OF PRUSSIA, PA 19406

ALABAMA, FLORIDA, GEORGIA, KENTUCKY, MISSISSIPPI, NORTH CAROLINA, PUERTO RICO, SOUTH CAROLINA, TENNESSEE, VIRGINIA, VIRGIN ISLANDS, OR WEST VIRGINIA, SEND APPLICATIONS TO:

U.S. NUCLEAR REGULATORY COMMISSION, REGION II
MATERIAL RADIATION PROTECTION SECTION
101 MARIETTA STREET, SUITE 2900
ATLANTA, GA 30323

IF YOU ARE LOCATED IN:

ILLINOIS, INDIANA, IOWA, MICHIGAN, MINNESOTA, MISSOURI, OHIO, OR WISCONSIN, SEND APPLICATIONS TO:

U.S. NUCLEAR REGULATORY COMMISSION, REGION III
MATERIALS LICENSING SECTION
799 ROOSEVELT ROAD
GLEN ELLYN, IL 60137

ARKANSAS, COLORADO, IDAHO, KANSAS, LOUISIANA, MONTANA, NEBRASKA, NEW MEXICO, NORTH DAKOTA, OKLAHOMA, SOUTH DAKOTA, TEXAS, UTAH, OR WYOMING, SEND APPLICATIONS TO:

U.S. NUCLEAR REGULATORY COMMISSION, REGION IV
MATERIAL RADIATION PROTECTION SECTION
611 RYAN PLAZA DRIVE, SUITE 1000
ARLINGTON, TX 76011

ALASKA, ARIZONA, CALIFORNIA, HAWAII, NEVADA, OREGON, WASHINGTON, AND U.S. TERRITORIES AND POSSESSIONS IN THE PACIFIC, SEND APPLICATIONS TO:

U.S. NUCLEAR REGULATORY COMMISSION, REGION V
MATERIAL RADIATION PROTECTION SECTION
1450 MARIA LANE, SUITE 210
WALNUT CREEK, CA 94596

PERSONS LOCATED IN AGREEMENT STATES SEND APPLICATIONS TO THE U.S. NUCLEAR REGULATORY COMMISSION ONLY IF THEY WISH TO POSSESS AND USE LICENSED MATERIAL IN STATES SUBJECT TO U.S. NUCLEAR REGULATORY COMMISSION JURISDICTION.

1. THIS IS AN APPLICATION FOR *(Check appropriate item)*

- [ ] A. NEW LICENSE
- [ ] B. AMENDMENT TO LICENSE NUMBER _____
- [X] C. RENEWAL OF LICENSE NUMBER 21-14161-01G

2. NAME AND MAILING ADDRESS OF APPLICANT *(Include Zip Code)*

Nuclear Diagnostics, Inc./
Sterling Biochemical, Inc.
575 Robbins Drive
Troy, MI 48083

3. ADDRESS(ES) WHERE LICENSED MATERIAL WILL BE USED OR POSSESSED.

575 Robbins Drive
Troy, MI 48083

8804220083 880929
REG3 LIC30
21-14161-01G PDR

4. NAME OF PERSON TO BE CONTACTED ABOUT THIS APPLICATION

James B. Smart, Ph.D.

TELEPHONE NUMBER: (313) 585-7600

SUBMIT ITEMS 5 THROUGH 11 ON 8½ x 11" PAPER. THE TYPE AND SCOPE OF INFORMATION TO BE PROVIDED IS DESCRIBED IN THE LICENSE APPLICATION GUIDE.

5. RADIOACTIVE MATERIAL
a. Element and mass number, b. chemical and/or physical form, and c. maximum amount which will be possessed at any one time. N/A

6. PURPOSE(S) FOR WHICH LICENSED MATERIAL WILL BE USED. N/A

7. INDIVIDUAL(S) RESPONSIBLE FOR RADIATION SAFETY PROGRAM AND THEIR TRAINING AND EXPERIENCE. N/A

8. TRAINING FOR INDIVIDUALS WORKING IN OR FREQUENTING RESTRICTED AREAS. N/A

9. FACILITIES AND EQUIPMENT. N/A

10. RADIATION SAFETY PROGRAM. N/A

11. WASTE MANAGEMENT. N/A

12. LICENSEE FEES *(See 10 CFR 170 and Section 170.31)*
FEE CATEGORY: 3K
AMOUNT ENCLOSED: $ 230.00

13. CERTIFICATION. *(Must be completed by applicant)* THE APPLICANT UNDERSTANDS THAT ALL STATEMENTS AND REPRESENTATIONS MADE IN THIS APPLICATION ARE BINDING UPON THE APPLICANT.

THE APPLICANT AND ANY OFFICIAL EXECUTING THIS CERTIFICATION ON BEHALF OF THE APPLICANT, NAMED IN ITEM 2, CERTIFY THAT THIS APPLICATION IS PREPARED IN CONFORMITY WITH TITLE 10, CODE OF FEDERAL REGULATIONS, PARTS 30, 32, 33, 34, 35, AND 40 AND THAT ALL INFORMATION CONTAINED HEREIN, IS TRUE AND CORRECT TO THE BEST OF THEIR KNOWLEDGE AND BELIEF.

WARNING: 18 U.S.C. SECTION 1001 ACT OF JUNE 25, 1948, 62 STAT. 749 MAKES IT A CRIMINAL OFFENSE TO MAKE A WILLFULLY FALSE STATEMENT OR REPRESENTATION TO ANY DEPARTMENT OR AGENCY OF THE UNITED STATES AS TO ANY MATTER WITHIN ITS JURISDICTION.

SIGNATURE–CERTIFYING OFFICER	TYPED/PRINTED NAME	TITLE	DATE
Cindy A. Wenzlick	Cindy A. Wenzlick	Supervisor, Technical Operations	6/10/87

14. VOLUNTARY ECONOMIC DATA

a. ANNUAL RECEIPTS
- [ ] < $250K
- [ ] $250K – 500K
- [ ] $500K – 750K
- [ ] $750K – 1M
- [ ] $1M – 3.5M
- [ ] $3.5M – 7M
- [ ] $7M – 10M
- [ ] > $10M

b. NUMBER OF EMPLOYEES *(Total for entire facility excluding outside contractors)*

c. NUMBER OF BEDS

d. WOULD YOU BE WILLING TO FURNISH COST INFORMATION *(dollar and/or staff hours)* ON THE ECONOMIC IMPACT OF CURRENT NRC REGULATIONS OR ANY FUTURE PROPOSED NRC REGULATIONS THAT MAY AFFECT YOU? *(NRC regulations permit it to protect confidential commercial or financial–proprietary–information furnished to the agency in confidence)*
- [ ] YES
- [ ] NO

FOR NRC USE ONLY

TYPE OF FEE	FEE LOG	FEE CATEGORY	COMMENTS	APPROVED BY
			RECEIVED JUN 16 1987 REGION III	
AMOUNT RECEIVED	CHECK NUMBER			DATE

RECEIVED JUN 16 1987 REGION III

PRIVACY ACT STATEMENT ON THE REVERSE

ATTACHMENT I

Kits distributed by NDI/SBI which are not manufactured by NDI/SBI

It is our understanding that the manufacturers of these kits listed below are in compliance with 10CFR 32.70.

MANUFACTURER: Farmos Diagnostica
Oulo, Finland

KITS: All kits contain I-125

-SEX HOROME BINDING GLOBULIN IMMUNORADIOMETRIC ASSAY KIT

-CORTISOL RADIOIMMUNOASSAY KIT

-EXTRACTION TESTOSTERONE RADIOIMMUNOASSAY KIT

-DIRECT TESTOSTERONE RADIOIMMUNOASSAY KIT

-EXTRACTION PROGESTERONE RADIOIMMUNOASSAY KIT

-PROGESTERONE RADIOIMMUNOASSAY KIT

-SPECTRIA DIRECT ESTRADIOL COATED TUBE RADIOIMMUNOASSAY KIT

-FERRITIN IRMA

-GLYCOCHOLIC ACID RADIOIMMUNOASSAY KIT

-DIRECT GLYCOCHOLIC ACID RADIOIMMUNOASSAY KIT

-GLYCOCHENODEOXYCHOLIC ACID RADIOIMMUNOASSAY

MANUFACTURER: Ciba Corning Diagnostics Corp.
Walpole, MA

KITS: All kits, except where indicated, contain I-125

-IMMOPHASE

-INSULIN RADIOIMMUNOASSAY

-TBG RADIOIMMUNOASSAY

-IF BLOCKING ANTIBODY (Co-57) RADIOASSAY

-LH RADIOIMMUNOASSAY

-HCG RADIOIMMUNOASSAY

-PROLACTIN RADIOIMMUNOASSAY

ATTACHMENT I (Cont.)

MANUFACTURER: Ciba Corning Diagnostics Corp.
Walpole, MA

KITS: All kits, except where indicated, contain I-125

-MAGIC

-TSH RADIOIMMUNOASSAY

-T3 RADIOIMMUNOASSAY

-T3 UPTAKE RADIOASSAY

-T4 RADIOIMMUNOASSAY

-FERRITIN RADIOIMMUNOASSAY

-CORTISOL RADIOIMMUNOASSAY

-DIGOXIN RADIOIMMUNOASSAY

-MAB TSH RADIOIMMUNOASSAY

-SINGLE STEP FT4 RADIOIMMUNOASSAY

-VITAMIN B12 (Co-57) FOLATE (^{125}I) RADIOASSAY

-VITAMIN B12/FOLATE NO-BOIL RADIOASSAY

MANUFACTURER: Monobind
Costa Mesa, CA

KIT: Contains I-125

-THYROTROPIN (TSH) SEQUENTIAL RIA TEST SYSTEM

MANUFACTURER: Becton Dickenson

KITS: Contain I-125

-BILE ACIDS SOLID PHASE COMPONENT SYSTEM

-FREE-T4 SOLID PHASE COMPONENT SYSTEM

MANUFACTURER: Wien Laboratories
Succasunna, NJ

KITS: Contains H-3

-17-HYDROXYPROGESTERONE TEST RIA CONTROL SERUM

ATTACHMENT I (Cont.)

MANUFACTURER: Diagnostic Products Corp.
Los Angeles, CA

KIT: Contains I-125

-DIGOXIN RIA KIT

ATTACHMENT II

KITS MANUFACTURED AND DISTRIBUTED BY NDI/SBI

All kits contain I-125, except the SPINSEP Fe59. Labelling for primary containers are shown in Attachment III.

1. SPINSEP T3U
2. TIPSEP T3U
3. MAAT-3
4. TRIA-PEG
5. TIPSEP-T4 RIA
6. LIQUA-T4 RIA
7. TETRIA-PEG
8. SPINSEP-TBG
9. TIPSEP-TBG
10. TIPSEP-DIGOXIN
11. SPINSEP Fe-59 (Contains Fe-59)

ATTACHMENT III

RADIOISOTOPE REAGENT LABELS

NDI

Nuclear Diagnostics, Inc.
575 Robbins, Troy, Michigan 48083

CAUTION
RADIOACTIVE
MATERIAL

Lot No.
Expires SAMPLE

TIPSEP™ T4 RIA
REAGENT-^{125}I

52 ml TIPSEP T4RIA REAGENT containing thyroxine ^{125}I, total activity less than 10 uCi, 60 days prior to expiration date, 0.07% ANS and a preservative.
Use with TIPSEP T4 RIA Test Kits According to Directions. For In Vitro Diagnostic Use Only.
STORE AT 2°-8°C
SL070 11-83

NDI

Nuclear Diagnostics, Inc.
575 Robbins, Troy, Michigan 48083

CAUTION
RADIOACTIVE
MATERIAL

Lot No.
Expires SAMPLE

TRIA™ T3RIA
REAGENT-^{125}I

55 ml TRIA T3RIA Reagent containing T3^{125}I, total activity less than 10uCi 60 days prior to expiration date, 0.07% ANS and a preservative.

Use with TRIA T3RIA Test Kits According to Directions. For In Vitro Diagnostic Use Only.
Store at 2°-8°C
SL013 6-82

NDI

Nuclear Diagnostics, Inc.
575 Robbins, Troy, Michigan 48084

CAUTION
RADIOACTIVE
MATERIAL

Lot No.
Expires SAMPLE

TETRIA™ T4RIA
REAGENT-^{125}I

55 ml TETRIA Reagent containing thyroxine ^{125}I, total activity less than 10 uCi 60 days prior to expiration date, 0.07% ANS and a preservative.

Use with TETRIA Test Kits According to Directions. For In Vitro Diagnostic Use Only

STORE AT 2°-8°C
SL002 5-82

NDI

Nuclear Diagnostics, Inc.
575 Robbins, Troy, MI 48083-4555

CAUTION
RADIOACTIVE
MATERIAL

LOT. NO. SAMPLE
EXPIRES

SPINSEP® T3U
REAGENT-^{125}I

105 ml SPINSEP T3U Reagent containing T3^{125}I, total activity less than 10 uCi, 60 days prior to expiration date.

Use with SPINSEP T3U Test Kits According to Directions. For In Vitro Diagnostic Use Only.

STORE AT 2-8°C.
4/87

NDI

Nuclear Diagnostics, Inc.
575 Robbins, Troy, Michigan 48084

CAUTION
RADIOACTIVE
MATERIAL

Lot No.
Expires SAMPLE

MAAT-3™
REAGENT-^{125}I

200 ml MAAT-3 Reagent containing T3^{125}I reacting pregated albumin (bovine), total activity less than 10 uCi 60 days prior to expiration date. Contains 0.05% Sodium Azide as a preservative.

Use with MAAT-3 Test Kits According to Directions. For In Vitro Diagnostic Use Only

Store at 2°-8°C
SL122 5-82

NDI

Nuclear Diagnostics, Inc.
575 Robbins, Troy, Michigan 48083

CAUTION
RADIOACTIVE
MATERIAL

Lot No.
Expires SAMPLE

TIPSEP™-TBG
REAGENT ^{125}I

100ml TIPSEP-TBG Reagent containing thyroxine ^{125}I in phosphate buffer. Total activity less than 10 uCi 60 days prior to expiration date.

Use with TIPSEP-TBG Test Kits According to Directions. For In Vitro Diagnostic use only.

STORE AT 2°-8°C
SL156 8-85

NDI

Nuclear Diagnostics, Inc.
575 Robbins, Troy, Michigan 48084

CAUTION
RADIOACTIVE
MATERIAL

Lot No.
Expires SAMPLE

TIPSEP-T3U™
REAGENT-^{125}I

105 ml TIPSEP T3U Reagent containing T3^{125}I, total activity approximately 10 uCi 60 days prior to expiration date

Use with TIPSEP T3U Test Kits According to Directions. For In Vitro Diagnostic Use Only

Store at 3°-8°C
(DO NOT FREEZE)
SL066

NDI

Nuclear Diagnostics, Inc.
575 Robbins, Troy, MI 48083-4555

CAUTION
RADIOACTIVE
MATERIAL

LOT. NO. SAMPLE
EXPIRES

SPINSEP®-TBG
REAGENT- ^{125}I

100 ml SPINSEP-TBG Reagent containing Thyroxine ^{125}I, total activity approximately 10 uCi, 60 days prior to expiration date

Use with SPINSEP-TBG Test Kits According to Directions For In Vitro Diagnostic Use

Store at 3°-8°C.
DO NOT FREEZE
3/87

NDI

Nuclear Diagnostics, Inc. Troy, Michigan 48084

CAUTION
RADIOACTIVE
MATERIAL

Lot
Expires
SL175 3/85

SAMPLE

TIPSEP™ DIGOXIN RIA
REAGENT-^{125}I

50ml TIPSEP DIGOXIN RIA REAGENT containing Digoxin (^{125}I), total activity less than 10 μCi 60 days prior to expiration date, prepared in phosphate buffer containing BSA and a preservative, pH 7.3.

Use with TIPSEP DIGOXIN RIA Test Kits according to directions. For In Vitro Diagnostic Use Only.

NDI

Nuclear Diagnostics, Inc. Troy, Michigan 48083

CAUTION
RADIOACTIVE
MATERIAL

Lot
Expires
Store at 2°-8°C SL-127 9/82

SAMPLE

LIQUA T4 RIA
REAGENT-^{125}I

44 ml. Liqua T_4 Reagent containing thyroxine (^{125}I), total activity less than 10 nCi 60 days prior to expiration date, 0.12 M TRIS-HCL Buffer, pH 7.8, with 1.56% Sodium Salicylate, Caution: 0.02% Sodium Azide as a preservative.

Use with LIQUA T_4RIA Test Kits according to directions. For In Vitro Diagnostic Use Only.

NDI

Nuclear Diagnostics, Inc. Troy, Michigan 48084

CAUTION
RADIOACTIVE
MATERIAL

Lot
Expires
Store at 2°-8°C

SAMPLE

MAAT-3™
REAGENT-^{125}I

50ml MAAT-3 Reagent containing T3 125I macroaggregated albumin (bovine), total activity less than 10μCi CI 60 days prior to expiration date. Contains 0.002% Sodium Azide as a preservative.

Use with MAAT-3 Test Kits According to Directions. For In Vitro Diagnostic Use Only.

NDI

Nuclear Diagnostics, Inc.
575 Robbins, Troy, Michigan 48084

CAUTION
RADIOACTIVE
MATERIAL

Lot No.
Expires

SAMPLE

SPINSEP® Fe^{59}
REAGENT

50 ml SPINSEP Fe^{59} REAGENT containing Fe^{59} as $FeCl_3$ in an acetic acid solution 0.02M. Total activity less than 20 μCi 60 days prior to expiration date.

Use with SPINSEP Fe^{59} Test Kits According to Directions. For In Vitro Diagnostic Use Only.

Store at Room Temperature

SL074 4-82

ATTACHMENT IV

WARNINGS & PRECAUTIONS FOR USE UNDER A GENERAL LICENSE

This Radioactive Material may be received, acquired, possessed and used only by physicians, veterinarians in the practice of veterinarian 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 general license of the U.S. Nuclear Regulatory Commission or a state with which the Commission has entered into an agreement for the exercise of regulatory authority.

1. Radioactive material should be stored in a specifically designed area in the original container, and all work should be carried out at designated work stations.

2. No pipetting of radioative materials should be done by mouth.

3. Do not eat or smoke within the desired work area, and wash your hands after working with radioactive material.

4. Spilled material should be cleaned up quickly and transferred to a suitable waste container. All contaminated areas should be washed with a suitable detergent solution.

5. Labeled material (^{125}I) contained in this kit may be disposed of via the normal sewer system if the concentration after dilution with laboratory discharge does not exceed 0.04 microcuries per liter (^{125}I) based on a daily average of effluent. Solid wastes may be disposed of through normal means. Deface all radiation labels before discarding.

6. Radioactive material, as described, is not for human use. Introdection into foods, beverages, cosmetics, drugs, or medicinals, or into products manufactured for commercial distributors is prohibited. Exempt quantities should not be combined. For IN VITRO diagnostics use.

ATTACHMENT V

WARNINGS AND PRECAUTIONS - SPECIFIC LICENSE

The following precautions must be observed in handling radioactive material:

1. Handling must preclude any pipetting by mouth.

2. There should be no smoking or eating while radioactive materials are being handled.

3. All laboratory tests should be conducted in a designated area of the laboratory.

4. Spills should be wiped up immediately and the contaminated materials placed in radioactive waste containers.

5. A complete description of proper waste disposal of radioactive wastes is covered in Title 10, Code of Federal Regulations, Part 20. SOLID WASTES (eg. used tubes, pipette tips, etc.) which cannot be or are not washed free of detectable radioactivity are most conveniently disposed of by transferring them to an agent authorized to dispose of radioactive materials. Such radioactive waste must be stored in a specially designated area in a covered metal or plastic container conspicuously marked with a radiation caution label. Solid waste which has been stored for decay until contamination no longer persists may be discarded as non-radioactive. However, many half lives are required for radioactive waste to decay to this extent. Since stored radioactive wastes contribute to the total possession of the licensee, the long term storage of such wastes reduces the amount of usable radioactive materials which can be on hand. LIQUID WASTE is to be discarded in accordance with your Specific License requirements.

6. Radioactive materials should be stored in a locked container or in a manner to preclude their accessibility to unauthorized personnel.

7. The use of radioisotopes should be confined to a designated area of the laboratory. Periodic surveys to detect contamination of these areas should be conducted.

8. It is advisable to store by-product material until used in their original shipping containers providing equivalent radiation protection.

NDI Nuclear Diagnostics Incorporated

SPINSEP-TBG

FOR THE QUANTITATIVE MEASUREMENT OF THYROXINE-BINDING PROTEIN CAPACITY IN SERUM

NDI Nuclear Diagnostics, Inc.

PROCEDURES

Add 100 µl of serum sample or control. Add stated volumes of standard. **1**

4 Vortex and incubate at room temperature for 15 minutes.

Dispense 1.0 ml of SPINSEP-TBG REAGENT into all tubes **2**

5 Centrifuge 10 minutes at 1250 x g

Add one SPINSEP TABLET to all tubes. Wait 5 minutes **3**

6 Decant, count

For further details of procedures consult package insert.

NDI

INTENDED USE

The SPINSEP™ TBG is a modified competitive protein binding analysis to measure the net effect of thyroxine-binding proteins in serum.

SUMMARY AND EXPLANATION OF TEST

The existence of thyroxine-binding proteins (TBP) in serum is well documented[1-3]. Upon entering the blood, approximately 99.98% of L-T4 and 99.64% of L-T3 become bound to thyroxine-binding proteins (TBP) in a reversible manner[4]. The hormones are transported throughout the body in the bound form. Clinical studies have shown that it is the concentration of the "free" thyroid hormone that determines the thyrometabolic status of an individual. The interaction between the thyroid hormones and their binding proteins (TBP) can be expressed by the equations:

$$T4 + TBP \rightleftharpoons TBP\text{-}T4$$

$$T3 + TBP \rightleftharpoons TBP\text{-}T3$$

As indicated by the equations, the concentration of "free" thyroid hormone is a function of both the serum thyroid hormone and TBP levels. Thus, a measure of total serum T4, T3 and TBP will provide an accurate assessment of the thyrometabolic status of the patient.

There are three major thyroxine-binding proteins: albumin, thyroxine-binding pre-albumin (TBPA), and thyroxine-binding globulin (TBG). Albumin has the weakest binding affinity for T4. Marked alterations in albumin concentration appear to have little or no effect on peripheral transport of thyroxine in normal patients. Thyroxine-binding pre-albumin has higher thyroxine-binding capacity but less binding affinity than TBG. Whether TBPA has a significant physiologic role in-vivo has been disputed[5]. Sterling[6] reported a less striking inverse correlation between the free thyroxine fraction with TBPA levels versus the free thyroxine fraction with TBG levels. Thyroxine-binding globulin is the most important of the three thyroxine binding proteins in human serum and has the strongest binding affinity for thyroxine. No known disease results from abnormal concentration of TBG[7]. When drugs, pregnancy, or illness cause alterations in the concentration of TBG, normal homeostatic mechanisms operate to increase or decrease the total thyroxine concentration so as to maintain a normal free T4 level.

PRINCIPLES OF TEST

This test measures primarily TBG binding capacity. The binding to TBPA is inhibited in the test procedure by the action of barbital buffer. Albumin contributes slightly to thyroxine binding measured by the SPINSEP-TBG procedure in patients with very low or absent TBG[8].

The principle of the test involves the saturation of binding sites with labeled T4. A very large excess of T4 in the reagent overwhelms the effect of the relatively small amount of endogenous serum T4.

The test is performed by adding 1 ml of a buffer solution containing labeled T4 to 0.1 ml of the patient serum. As the quantity of added TBG (from patient serum or TBG standard serum) increases, the fraction of free T4 (not bound to TBG) decreases. Thus, the TBG concentration (in ug/dl T4 Binding Capacity) of a sample can be determined by measuring the distribution of the labeled T4 and referring to a standard curve. The standard curve is a plot of the TBG concentration (in ug/dl Binding Capacity) versus the radioactivity (T4 bound to TBG or T4 free from TBG).

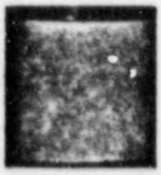
The distribution of TBG-bound versus TBG-free T4 ligand is determined by centrifugation of the reaction mixture with NDI-SPINSEP Tablets. The resin tablets contain precisely measured quantities of specially-processed, high quality ion exchange resin. When the reaction mixture comes in contact with the resin, free T4 (not bound to TBG) adheres to the resin and is centrifuged to the bottom of the tube while the bound T4 (bound to TBG) remains free.

Since the total activity added to the reaction mixture can be determined and does not change from sample to sample, the distribution of radioactivity (bound versus free) can be obtained by measuring the radioactivity content of the centrifuged pellet.

The amount of free T4 (labeled and unlabeled) which binds to the resin varies inversely with the TBG content of the serum. Thus, when the serum TBG concentration is high, a small percentage of labeled T4 is "free" to bind to the resin and vice versa.

REAGENTS

1. **SPINSEP-TBG REAGENT-^{125}I**, 105 ml, containing thyroxine I-125, less than 10 uCi sixty days to expiration date, in a barbital buffer. Store at 2°-8°C.



2. **SPINSEP TABLETS,** 100 tablets, containing ion exchange resin (1-15%) in a matrix. Store at room temperature.
3. Two vials lyophilized human serum standard. Assay value is given on the enclosed circular. Store at 2°-8°C.

Warnings and Precautions

WARNING. This radioactive material may be received, acquired, possessed and used only by physicians, 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 license of the U.S. Nuclear Regulatory Commission or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority. For In Vitro Diagnostic Use Only.

NUCLEAR DIAGNOSTICS, INC.

The following precautions must be observed in handling radioactive material:

1. Handling must preclude any pipetting by mouth.
2. There should be no smoking or eating while radioactive materials are being handled.
3. All laboratory tests should be conducted in a designated area of the laboratory.
4. Spills should be wiped up immediately and the contaminated materials placed in radioactive waste containers.
5. A complete description of proper waste disposal of radioactive wastes is covered in C.F.R. Title 10, Code of Federal Regulations, Part 20. SOLID WASTES (eg. used tubes, pipette tips, etc.) which cannot be or are not washed free of detectable radioactivity are most conveniently disposed of by transferring them to an agent authorized to dispose of radioactive materials. Such radioactive waste must be stored in a specially designated area in a covered metal or plastic container conspicuously marked with a radiation caution label. Solid waste which has been stored for decay until contamination no longer persists may be discarded as non-radioactive. However, many half lives are required for radioactive waste to decay to this extent. Since stored radioactive wastes contribute to the total possession of the licensee, the long term storage of such wastes reduces the amount of usable radioactive materials which can be on hand. LIQUID WASTE is to be discarded into a sanitary sewer and should be handled with appropriate care so as to avoid contamination of personnel and work areas. It is advisable to flush the radioactive materials with ample running water. A specific sink should be designated for disposal of radioactive liquids.
6. Radioactive materials should be stored in a locked container or in a manner to preclude their accessibility to unauthorized personnel.
7. The use of radioisotopes should be confined to a designated area of the laboratory. Periodic surveys to detect contamination of these areas should be conducted.
8. General licensees are advised to store by-product material until used in their original shipping container providing equivalent radiation protection.

WARNING - POTENTIAL BIOHAZARDOUS MATERIAL

Each unit of source materials used in the preparation of these products has been tested and found non-reactive for the presence of hepatitis B surface antigen (HB_sAg) by counter-electrophoresis or radioimmunoassay. However due to the possibility that the causative agent of viral hepatitis may still be present, it is recommended that these products be handled in the same manner as any potentially infective biological material such as clinical specimens.

STORAGE PRECAUTIONS

Alteration in the physical appearance of the reagents or in the slope of the standard curve, or values of control sera outside the acceptable range may be an indication of reagent instability. Components stored according to directions may be used up to the expiration date stated on their labels. TBG standard serum is stable prior to reconstitution throughout expiration date stated on their label. Once reconstituted, the TBG standard is stable for three days at 2°-8°C. Avoid freezing of the reagent since this may cause precipitation of the buffer.

SPECIMEN COLLECTION AND PREPARATION

No specific preparation of the patient is required. Sera are collected in the conventional manner with no additives or preservatives needed. Sera should

be refrigerated if they are to be tested within 48 hours. Otherwise, sera should be frozen and thawed immediately before testing. Mix thawed serum samples well before testing. Avoid repeated cycles of freezing and thawing.

PROCEDURE

Materials Provided

1. SPINSEP-TBG REAGENT-^{125} I
2. SPINSEP TABLETS
3. SPINSEP-TBG Serum Standard
4. Disposable Test Tubes (Available with Cat. No. 17100)
5. Graph Paper

Materials Required But Not Provided

1. Semi-automatic pipettes and disposable tips, volumes of 50, 100, 200, and 300 µl.
2. Semi-automatic dispensor, 1.0 ml
3. Test tube rack
4. Distilled or deionized water
5. Volumetric pipette
6. Scintillation well counter capable of detecting I-125
7. Vortex mixer
8. Centrifuge capable of producing 1250 x g force

Reconstitution of Lyophilized Standard

1. Using volumetric pipette, reconstitute the lyophilized serum with the stated label volume of deionized or distilled water.
2. Allow a minimum of 30 minutes for complete reconstitution and mix by gentle inversion before use. Avoid creating a foam.
3. If not used immediately, it is suggested that the serum be aliquoted and frozen until needed.
4. Mix thawed samples well before use; do not vortex.

Stability of Standard

... To expiration date on vial when stored at 2°-8°C in the dried state.
... Three days at 2°-8°C after reconstitution.
... Sixty days after reconstitution when stored frozen.

Details of Procedure

1. Bring all reagents, samples, standard and controls to room temperature. Mix gently, but thoroughly.
2. ADD 100 µl of patient serum or control into designated tubes. The standard curve is obtained by pipetting 50, 100, 200 and 300 µl of standard serum into appropriate tubes.
3. DISPENSE 1.0 ml of SPINSEP-TBG REAGENT into all tubes. Add 1.0 ml of reagent into two tubes labeled TOTAL COUNTS, set aside for counting.
4. ADD one SPINSEP TABLET into each tube. Wait five (5) minutes.
5. Vortex tubes vigorously (10 seconds) and incubate at room temperature for a minimum of 15 minutes (maximum 30 minutes).
6. Centrifuge tubes at a minimum of 1250 x g for 10 minutes at room temperature.
7. Decant the supernatant and drain tubes well. Remove last drop of liquid by touching the lip of test tubes on an absorbent surface.

RESULTS

Counting and Calculations

1. Set scintillation well counter to detect I-125 according to manufacturer's recommendations.
2. Determine the radioactivity remaining in each tube to desired level of counting accuracy. A counting time of 0.5 minutes should suffice for a counting error approximately 1.0% or less (i.e. total counts collected of 10,000 or more).
3. Calculate the % Resin Bound by:

$$\% \text{ Resin Bound} = \frac{\text{Avg count of Patient or Standard Tube}}{\text{Total Count of 1 ml Reagent}} \times 100$$

SAMPLE DATA
(Not to be used in lieu of Actual Data)

Sample	Avg. Counts	% Resin Bound	TBG Value µg % T4 B.C.*
Total Count	55568	—	—
50 µl Standard	45111	81.2	9.0
100 µl Standard	39496	71.1	18.0
200 µl Standard	29692	53.4	36.0
300 µl Standard	22551	40.6	54.0
Patient A	39417	70.9	18.5

* µg % T4 Binding Capacity

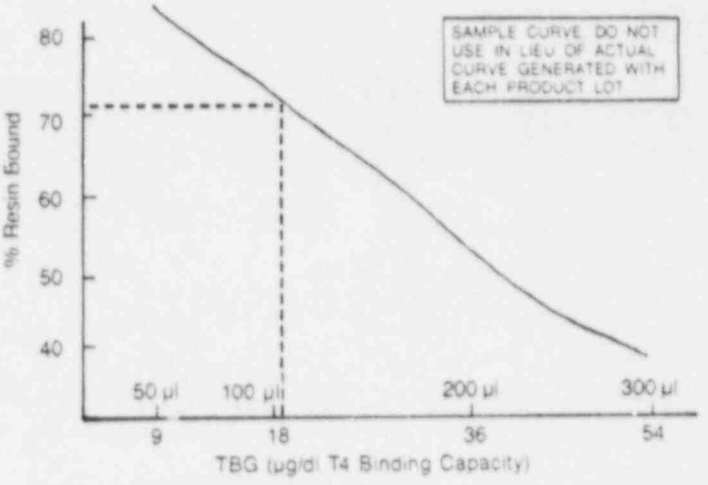
Preparation of Standard Curve and Determination of Final Results

1. On the SPINSEP-TBG graph paper provided, plot the % Resin Bound obtained for 50, 100, 200, and 300 µl of standard serum versus the amount of TBG added. The amount of TBG added is as follows:

µl added	TBG added	
50	0.5	
100	1.0	
200	2.0	X TBG value of standard*
300	3.0	

*The TBG value of the standard serum is given on the enclosed circular.

2. Using a French curve, draw a "best fit" curve through the plotted values of the standard results obtained.
3. Determine the TBG concentration (in µg/dl T4 binding capacity) in unknown samples by extending a horizontal line from the % Resin Bound (Y axis) to the point where the line intersects the curve. At this point, extend a vertical line to the X axis and read the value for the unknown.



QUALITY CONTROL PROCEDURES

Control sera should be included on a routine basis with each group of sera assayed. Additional control sera may be obtained by pooling selected groups of patient sera or from commercial sources. It is preferable to have controls for each of the three categories of thyroid status.

The laboratory should periodically calculate the coefficient of variation (CV) of test results obtained from control sera tested over a period of time. After an accepted CV has been established, marked deviations from the acceptable limits may suggest problems with the test procedure (technician error, reagent deterioration, equipment malfunction, etc.). The establishment of control limits is discussed in most general laboratory handbooks.[11-13]

LIMITATIONS OF PROCEDURE

Some of the factors known to increase TBG and total T4 concentrations are: pregnancy, estrogens, anti-ovulatory drugs, genetic factors, hepatic disease (elevated estrogen levels), and hyperproteinemia. Factors known to decrease TBG and total T4 concentrations are: androgens, nephrosis with hypoproteinemia, genetic factors and hepatic disease (decreased protein synthesis). Drugs known to result in low serum T4 levels due to displacement of T4 from binding proteins are: dilantin and massive doses of salicylates.[10, 14]

EXPECTED VALUES

The suggested normal range for the SPINSEP-TBG procedure is 15-25 µg/dl T4 binding capacity.

It is suggested that each laboratory establish its own normal range due to unique factors which may govern the thyroxine-binding globulin levels in their particular geographic area. The normal range of 15-25 µg/dl T4 binding capacity was established based on a study of 131 normal hospital employees. Ten euthyroid sera of females taking estrogens or birth control pills had a mean TBG value of 30.4 with range of 26.0-41.0 µg/dl T4 binding capacity. None of these values fell within the normal range. The SPINSEP-TBG values for hyperthyroid and hypothyroid patient groups were 20.3 and 22.9 µg/dl T4 binding capacity, respectively[9].

PERFORMANCE CHARACTERISTICS

Effect of Added Thyroxine

To demonstrate that the test is not affected by the amount of endogenous T4 present in the serum sample, known quantities of thyroxine were added to a base serum tested by the SPINSEP-TBG procedure. The results are summarized below.

Table 1

Base Serum TBG Level (µg/dl T4 B.C.)	Added T4 (µg/dl) to Base Serum	Measured TBG Level (µg/dl T4 B.C.)	Percent Change From Base Serum
20.2	5	21.0	+4.0
	10	20.5	+1.5
	15	20.0	-1.0
	20	20.0	-1.0

Sensitivity

The sensitivity of the SPINSEP-TBG test procedure is approximately 0.4 µg/dl T4 binding capacity.

Effect of Added Albumin

There is evidence that albumin contributes partially to the thyroxine binding measured in the test. However, the effect is only shown in patients with very low or absent TBG[15]. Results of a study are summarized below.

	Albumin Added to Base (% of Normal Albumin)		
	0%	50%	100%
Low TBG	9.0	12.3	15.8
Normal TBG	20.5	20.9	22.3
High TBG	28.9	29.9	31.9

Precision

Frozen pooled sera were employed to determine the intra- and inter-assay precision of the SPINSEP-TBG procedure. The sera were assayed in duplicate over several days.

Intra-assay Precision (in duplicate)

	Mean Value (µg/dl T4 B.C.)	S.D. (µg/dl T4 B.C.)	Coefficient of Variation (%)	# of Assays
Pool #1	16.62	1.35	3.1	20
Pool #2	20.26	0.87	2.2	20

Inter-assay Precision (in duplicate)

	Mean Value (µg/dl T4 B.C.)	S.D. (µg/dl T4 B.C.)	Coefficient of Variation (%)	# of Assays
Pool #1	16.62	1.35	8.1	20
Pool #2	20.26	0.87	4.3	20

Inter-assay precision of the test is indicated by the coefficient of variation for a sample assayed on different days.

Intra-assay precision is obtained by determining the mean of the coefficients of variation for the duplicate determinations of serum.

Test Tubes

It is suggested that disposable plastic test tubes (12 x 75 mm) be used.

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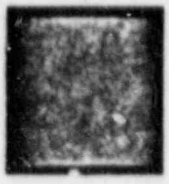

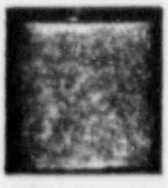

ORDERING INFORMATION

SPINSEP®- TBG

CAT. NO. 17100 100 Test Kit
CAT. NO. 17111 100 Test Kit (without tubes)

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