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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

L 00139
11757

December 8, 1975

Nuclear Regulatory Commission
Washington, D.C. 20555
Attn.: Mr. Douglas Collins

Gentlemen:

We hereby wish to apply for a specific license, pursuant to 10 CFR § 32.71, to manufacture or distribute by-product material for use under the general license of 10 CFR § 31.11 for the following products, all of which contain Iodine-125 not exceeding 10 microcuries in each prepackaged unit.

1. Thyrostat-4/FTI, 25 test kit, List 09126
2. Thyrostat-4/FTI, 100 test kit, List 09151
3. Thyrostat-4/FTI, 5 x 100 test kits, List 09171
4. Thyrostat-FTI, 25 test kit, List 09127
5. Thyrostat-FTI, 100 test kit, List 09152
6. Thyrostat-FTI, 5 x 100 test kits, List 09172
7. Thyrostat-3, 25 test kit, List 09026
8. Thyrostat-3, 100 test kit, List 09028
9. Thyrostat-3, 5 x 100 test kits, List 09027
10. Digoxin Immutope Kit, 100 test kit, List 09350
11. Digoxin Immutope Kit, 200 test kit, List 09360
12. Gastrin Immutope Kit, 100 test kit, List 09510

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 outlined in the attached brochures.

In addition, a check for $335.00 is enclosed to cover the appli-

RECEIVER: DRA:BMB
Date 12-15-75
Time 8 AM
By mcs
From folder

COPIES SENT TO OFF. OF INSPECTION AND ENFORCEMEN

Applicant
Check No. 438345
Amount $335-00
Date of Check 12-4-75
Date Check Rec'd 12-15-75
Received By mcs

A/2

59649

see 59057 for audit

cation fee.

We trust that this information will be adequate and look forward to your issuing this license.

Sincerely,

C L Kroll

C. L. Kroll

SQUIBB REFRIGERATE AT 2° to 8°C.

50 ml. List 09126

THYROSTAT®-4/FTI BUFFER SOLUTION

THYROXINE BINDING GLOBULIN/T4 I 125 BUFFER SOLUTION

FOR LABORATORY USE ONLY with THYROSTAT-4/FTI Adsorbent Tablets • See accompanying directions

For *in vitro* Diagnostic Use • **Shake well before using**

Not for Internal or External Use in Humans or Animals

Total Act. <2.6 microcuries As of _____ Noon E.S.T.

LOT NO. _____ EXP. DATE _____

E. R. Squibb & Sons, Inc. Princeton, N.J. 08540 Made in U.S.A. M2379

SQUIBB

CAUTION RADIOACTIVE MATERIAL

25 TEST KIT List 09126

THYROSTAT®-4/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:

25 plastic test tubes
1 vial (28 tabs.) THYROSTAT-4/FTI ADSORBENT TABLETS
1 bottle (50 ml.) THYROSTAT-4/FTI I 125 BUFFER SOLUTION <2.6 microcuries per bottle
1 vial (3.0 ml.) THYROSTAT-4/FTI CONTROL SERUM
1 vial (25 ml.) THYROSTAT-4/FTI EXTRACTION ALCOHOL
4 vials (1.2 ml. ea.) THYROSTAT-4 STANDARD SOLUTION (1 each of 0 μg., 6 μg., 12 μg., and 18 μg.)
4 THYROSTAT-4 TOTAL COUNT RESIN TUBES

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

REFRIGERATE AT 2° to 8°C.

RADIOACTIVE MATERIAL: Gamma radiation at surface of parcel less than 10 milliroentgens for 24 hours — No significant Alpha, Beta or Neutron radiation

LOT NO.:
EXP. DATE:

E. R. Squibb & Sons, Inc., Princeton, N.J. 08540 Made in U.S.A. M7866

59649

SQUIBB REFRIGERATE AT 2° to 8°C.

200 ml. List 09151

THYROSTAT®-4/FTI BUFFER SOLUTION

THYROXINE BINDING GLOBULIN/T4 I 125 BUFFER SOLUTION

FOR LABORATORY USE ONLY with THYROSTAT-4/FTI Adsorbent Tablets • See accompanying directions

For *in vitro* Diagnostic Use • **Shake well before using**

Not for Internal or External Use in Humans or Animals

Total Act. <9.7 microcuries As of _____ Noon E.S.T.

LOT NO. _____ EXP. DATE _____

E. R. Squibb & Sons, Inc.
Princeton, N.J. 08540 Made in U.S.A. M2381

SQUIBB CAUTION RADIOACTIVE MATERIAL Medotopes®

100 TEST KIT

THYROSTAT®-4/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

REFRIGERATE AT 2° to 8°C.

CONTENTS: List 09151

100 plastic test tubes
1 vial (105 tabs.) THYROSTAT-4/FTI ADSORBENT TABLETS
1 bottle (200 ml.) THYROSTAT-4/FTI I 125 BUFFER SOLUTION <9.7 microcuries per bottle
1 vial (3.0 ml.) THYROSTAT-4/FTI CONTROL SERUM
1 vial (100 ml.) THYROSTAT-4/FTI EXTRACTION ALCOHOL
4 vials (1.2 ml. ea.) THYROSTAT-4 STANDARD SOLUTION (1 each of 0 μg., 6 μg., 12 μg., and 18 μg.)
8 THYROSTAT-4 TOTAL COUNT RESIN TUBES

LOT NO.:
EXP. DATE:

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

RADIOACTIVE MATERIAL: Gamma radiation at surface of parcel less than 10 milliroentgens for 24 hours — No significant Alpha, Beta or Neutron radiation

E. R. Squibb & Sons, Inc.
Princeton, N.J. 08540 Made in U.S.A. M7870

59649

SQUIBB REFRIGERATE AT 2° to 8°C. CAUTION RADIOACTIVE MATERIAL

200 ml. List 09151

THYROSTAT®-4/FTI BUFFER SOLUTION

THYROXINE BINDING GLOBULIN/T4 I 125 BUFFER SOLUTION

FOR LABORATORY USE ONLY with THYROSTAT-4/FTI Adsorbent Tablets • See accompanying directions

For *in vitro* Diagnostic Use • **Shake well before using**

Not for Internal or External Use in Humans or Animals

Total Act. <9.7 microcuries As of _____ Noon E.S.T.

LOT NO. _____ EXP. DATE _____

E. R. Squibb & Sons, Inc.
Princeton, N.J. 08540 Made in U.S.A. M2381

SQUIBB CAUTION RADIOACTIVE MATERIAL Medotopes®

500 TEST KIT

THYROSTAT®-4/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

REFRIGERATE AT 2° to 8° C.

CONTENTS: List 09171

500 plastic test tubes
- 5 vials (105 tabs. each) THYROSTAT-4/FTI ADSORBENT TABLETS
- 5 bottles (200 ml. each) THYROSTAT-4/FTI I 125 BUFFER SOLUTION <9.7 microcuries per bottle
- 5 vials (3.0 ml. each) THYROSTAT-4/FTI CONTROL SERUM
- 5 vials (100 ml. each) THYROSTAT-4/FTI EXTRACTION ALCOHOL
- 20 vials (1.2 ml. ea.) THYROSTAT-4 STANDARD SOLUTION (5 each of 0 μg., 6 μg., 12 μg., and 18 μg.)
- 40 THYROSTAT-4 TOTAL COUNT RESIN TUBES

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

RADIOACTIVE MATERIAL: Gamma radiation at surface of parcel less than 10 milliroentgens for 24 hours — No significant Alpha, Beta or Neutron radiation

LOT NO.:
EXP. DATE:

E. R. Squibb & Sons, Inc.
Princeton, N.J. 08540 Made in U.S.A. M7871

SQUIBB

J3-414

THYROSTAT®-4/FTI DIAGNOSTIC TEST KIT

For Quantitative Measurement of Total Serum Thyroxine or Free Thyroxine Index for the Evaluation of Thyroid Function

For *In Vitro* Diagnostic Use
For Professional Use Only

PRINCIPLES OF THE TEST

Total Serum Thyroxine

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. 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 a specific thyroxine-binding protein, a radiolabeled derivative of thyroxine, and a purified sample of thyroxine to serve as a reference standard. 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 protein 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 absolute quantity of thyroxine (T_4) in a serum sample is determined from a standard curve prepared with known amounts of a thyroxine standard preparation. In the Thyrostat-4 test, the thyroxine is extracted from serum with ethanol and the alcoholic extract is mixed with ^{125}I-labeled thyroxine that is bound to thyroxine-binding proteins. An organic adsorbent is used to separate bound thyroxine from free thyroxine.

When the thyroid gland produces an excess of thyroid hormones, as in hyperthyroidism, the concentration of T_4 in the serum will be elevated, and a greater proportion of the radiolabeled thyroxine will be bound to the added adsorbent. Conversely, when the thyroid hormone production is decreased, as in hypothyroidism, the concentration of T_4 in the serum will be decreased, and a smaller proportion of the radiolabeled thyroxine will be bound to the added adsorbent.

Free Thyroxine Index

In the absence of abnormalities in serum 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

The Thyrostat-4 *in vitro* diagnostic test for the measurement of total serum thyroxine is based on the classic Murphy-Pattee "competitive protein binding analysis" procedure, recently redesignated "radiotransin assay." It has been suggested by an *ad hoc* committee of the American Thyroid Association[1] that this method of measuring thyroxine concentration 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_4RT_3 is the mathematical product of the results of the T_4(D) and RT_3U tests.

J3-414

SQUIBB REFRIGERATE AT 2° to 8°C.

50 ml. List 09126

THYROSTAT®-4/FTI BUFFER SOLUTION

THYROXINE BINDING GLOBULIN/T4 I 125 BUFFER SOLUTION

FOR LABORATORY USE ONLY with THYROSTAT-4/FTI Adsorbent Tablets • See accompanying directions

For *in vitro* Diagnostic Use • **Shake well before using**

Not for Internal or External Use in Humans or Animals

Total Act. <2.6 microcuries As of _____ Noon E.S.T.

LOT NO. _____ EXP. DATE _____

E. R. Squibb & Sons, Inc.
Princeton, N.J. 08540 Made in U.S.A. M2379

SQUIBB

CAUTION
RADIOACTIVE MATERIAL

25 TEST KIT List 09127

THYROSTAT®-FTI DIAGNOSTIC TEST KIT

LOT NO.:
EXP. DATE:

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:
- 25 plastic test tubes
- 1 vial (28 tabs.) THYROSTAT-4/FTI ADSORBENT TABLETS
- 1 bottle (50 ml.) THYROSTAT-4/FTI I 125 BUFFER SOLUTION <2.6 microcuries per bottle
- 1 vial (3.0 ml.) THYROSTAT-4/FTI CONTROL SERUM
- 1 vial (25 ml.) THYROSTAT-4/FTI EXTRACTION ALCOHOL

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

REFRIGERATE AT 2° to 8°C.

RADIOACTIVE MATERIAL: Gamma radiation at surface of parcel less than 10 milliroentgens for 24 hours — No significant Alpha, Beta or Neutron radiation

E. R. Squibb & Sons, Inc., Princeton, N.J. 08540 Made in U.S.A. M7867

SQUIBB REFRIGERATE AT 2° to 8°C.

200 ml. List 09151

THYROSTAT®-4/FTI BUFFER SOLUTION

THYROXINE BINDING GLOBULIN/T4 I 125 BUFFER SOLUTION

FOR LABORATORY USE ONLY with THYROSTAT-4/FTI Adsorbent Tablets • See accompanying directions

For *in vitro* Diagnostic Use • **Shake well before using**

Not for Internal or External Use in Humans or Animals

Total Act. <9.7 microcuries As of _____ Noon E.S.T.

LOT NO. _____ EXP. DATE _____

E. R. Squibb & Sons, Inc.
Princeton, N.J. 08540 Made in U.S.A. M2381

SQUIBB

CAUTION
RADIOACTIVE MATERIAL

Medotopes®

100 TEST KIT

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

REFRIGERATE AT 2° to 8° C.

CONTENTS: List 09152

100 plastic test tubes
1 vial (105 tabs.) THYROSTAT-4/FTI ADSORBENT TABLETS
1 bottle (200 ml.) THYROSTAT-4/FTI I 125 BUFFER SOLUTION <9.7 microcuries per bottle
1 vial (3.0 ml.) THYROSTAT-4/FTI CONTROL SERUM
1 vial (100 ml.) THYROSTAT-4/FTI EXTRACTION ALCOHOL

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

RADIOACTIVE MATERIAL: Gamma radiation at surface of parcel less than 10 milliroentgens for 24 hours — No significant Alpha, Beta or Neutron radiation

LOT NO.:
EXP. DATE:

E. R. Squibb & Sons, Inc.
Princeton, N.J. 08540 Made in U.S.A. M7868

SQUIBB REFRIGERATE AT 2° to 8°C.

200 ml. List 09151

THYROSTAT®-4/FTI BUFFER SOLUTION

THYROXINE BINDING GLOBULIN/T4 I 125 BUFFER SOLUTION

FOR LABORATORY USE ONLY with THYROSTAT-4/FTI Adsorbent Tablets • See accompanying directions

For *in vitro* Diagnostic Use • **Shake well before using**

Not for Internal or External Use in Humans or Animals

Total Act. <9.7 microcuries As of _____ Noon E.S.T.

LOT NO. _____ EXP. DATE _____

E. R. Squibb & Sons, Inc.
Princeton, N.J. 08540 Made in U.S.A. M2381

SQUIBB CAUTION RADIOACTIVE MATERIAL Medotopes®

500 TEST KIT

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

REFRIGERATE AT 2° to 8°C.

CONTENTS: List 09172

500 plastic test tubes
5 vials (105 tabs. each) THYROSTAT-4/FTI ADSORBENT TABLETS
5 bottles (200 ml.each) THYROSTAT-4/FTI I 125 BUFFER SOLUTION <9.7 microcuries per bottle
5 vials (3.0 ml.each) THYROSTAT-4/FTI CONTROL SERUM
5 vials (100 ml.each) THYROSTAT-4/FTI EXTRACTION ALCOHOL

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

RADIOACTIVE MATERIAL: Gamma radiation at surface of parcel less than 10 milliroentgens for 24 hours — No significant Alpha, Beta or Neutron radiation

LOT NO.:
EXP. DATE:

E. R. Squibb & Sons, Inc.
Princeton, N.J. 08540 Made in U.S.A. M7869

SQUIBB

J3-415

THYROSTAT®-FTI DIAGNOSTIC TEST KIT

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 "radiotracer in assay" be identified as "Thyroxine (displacement)," abbreviated "T_4(D)," and expressed as micrograms per 100 milliliters of serum (μg./100 ml.).

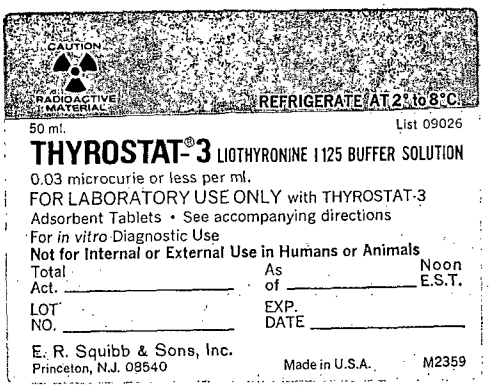
When there are pronounced alterations in the binding capacity of the transport proteins, the T_4(D) test, used in conjunction with the commonly used T_3 test (Resin Triiodothyronine uptake-RT_3U) provides an indirect measure of the concentration of free T_4, which is highly correlated with the thyroid 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*

J3-415



SQUIBB

CAUTION
RADIOACTIVE MATERIAL

25 TEST KIT

List 09026

THYROSTAT®-3 DIAGNOSTIC TEST KIT with CONTROL PAK

LOT NO.:
EXP. DATE:

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:

25 plastic test tubes
1 vial (28 tabs.) THYROSTAT-3 ADSORBENT TABLETS
1 bottle (50 ml.) THYROSTAT-3 LIOTHYRONINE I 125 BUFFER SOLUTION
0.03 microcurie or less per ml.
1 THYROSTAT-3 CONTROL PAK (Each CONTROL PAK contains
1 vial (1.0 ml.) THYROSTAT-3 NORMAL CONTROL SERUM;
1 vial (1.0 ml.) THYROSTAT-3 HYPER CONTROL SERUM;
4 THYROSTAT-3 TOTAL COUNT RESIN TUBES)

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

REFRIGERATE AT 2° to 8°C.

RADIOACTIVE MATERIAL: Gamma radiation at surface of parcel less than 10 milliroentgens for 24 hours — No significant Alpha, Beta or Neutron radiation

E. R. Squibb & Sons, Inc., Princeton, N.J. 08540 Made in U.S.A. M7742

59649

CAUTION
RADIOACTIVE MATERIAL

REFRIGERATE AT 2° to 8° C.

200 ml. List 09028

THYROSTAT®-3 LIOTHYRONINE I 125 BUFFER SOLUTION

0.03 microcurie or less per ml.

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

For *in vitro* Diagnostic Use

Not for Internal or External Use in Humans or Animals

Total Act. _____ As of _____ Noon E.S.T.

LOT NO. _____ EXP. DATE _____

E. R. Squibb & Sons, Inc.
Princeton, N.J. 08540 Made in U.S.A. M7756

SQUIBB

CAUTION
RADIOACTIVE MATERIAL

Medotopes®

100 TEST KIT

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

REFRIGERATE AT 2° to 8° C.

CONTENTS: List 09028

100 plastic test tubes
- 1 vial (105 tablets) THYROSTAT-3 ADSORBENT TABLETS
- 1 bottle (200 ml.) THYROSTAT-3 LIOTHYRONINE I 125 BUFFER SOLUTION 0.03 microcurie or less per ml.
- 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

RADIOACTIVE MATERIAL: Gamma radiation at surface of parcel less than 10 milliroentgens for 24 hours—No significant Alpha, Beta or Neutron radiation

LOT NO.:
EXP. DATE:

E. R. Squibb & Sons, Inc.
Princeton, N.J. 08540 Made in U.S.A. M7755

CAUTION
RADIOACTIVE MATERIAL

REFRIGERATE AT 2° to 8° C.

200 ml. List 09028

THYROSTAT®-3 LIOTHYRONINE I 125 BUFFER SOLUTION

0.03 microcurie or less per ml.

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

For in vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals

Total Act._____ As of_____ Noon E.S.T.

LOT NO._____ EXP. DATE_____

E. R. Squibb & Sons, Inc.
Princeton, N.J. 08540 Made in U.S.A. M7756

SQUIBB®

CAUTION
RADIOACTIVE MATERIAL

Medotopes®

500 TEST KIT

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

REFRIGERATE AT 2° to 8° C.

CONTENTS: List 09027

500 plastic test tubes
- 5 vials (105 tablets each) THYROSTAT-3 ADSORBENT TABLETS
- 5 bottles (200 ml. each) THYROSTAT-3 LIOTHYRONINE I 125 BUFFER SOLUTION 0.03 microcurie or less per ml.
- 5 vials (1.0 ml. each) THYROSTAT-3 NORMAL CONTROL SERUM
- 5 vials (1.0 ml. each) THYROSTAT-3 HYPER CONTROL SERUM
- 40 THYROSTAT-3 TOTAL COUNT RESIN TUBES

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

RADIOACTIVE MATERIAL: Gamma radiation at surface of parcel less than 10 milliroentgens for 24 hours—No significant Alpha, Beta or Neutron radiation

LOT NO.:
EXP. DATE:

E. R. Squibb & Sons, Inc.
Princeton, N.J. 08540 Made in U.S.A. M7762

SQUIBB

J3-358

THYROSTAT®-3 DIAGNOSTIC TEST KIT

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 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 days. The isotope decays in a complex fashion with emission of x-rays and gamma rays whose radiation energies are 27.5 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-, 100-, and 500-test kits.

The Thyrostat-3 25-test kit provides 25 plastic test tubes, 1 vial of Adsorbent Tablets (25), 1 bottle of Liothyronine I 125 Buffer Solution (50 ml., containing 0.03 μCi or less per ml.), 1 Control Pak containing 1 vial (1.0 ml.) Normal Control Serum, 1 vial (1.0 ml.) Hyper Control Serum, and 4 Total Count Resin Tubes.

The Thyrostat-3 100-test kit provides 100 plastic test tubes, 1 vial of Adsorbent Tablets (105), 1 bottle of Liothyronine I 125 Buffer Solution (200 ml., containing 0.03 μCi or less per

J3-358

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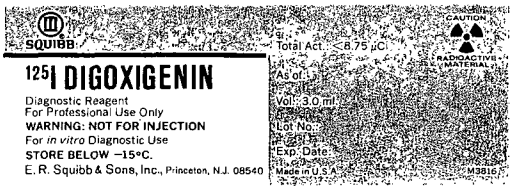


^{125}I DIGOXIGENIN

Diagnostic Reagent
For Professional Use Only
WARNING: NOT FOR INJECTION
For *in vitro* Diagnostic Use
STORE BELOW −15°C.
E. R. Squibb & Sons, Inc., Princeton, N.J. 08540

DIGOXIN IMMUTOPE® Kit List 09350

Diagnostic Reagent • For Professional Use Only
For *in vitro* Diagnostic Use • **WARNING: NOT FOR INJECTION**
Contains sufficient material for 100 radioimmunoassay tests
Not for Internal or External Use in Humans or Animals

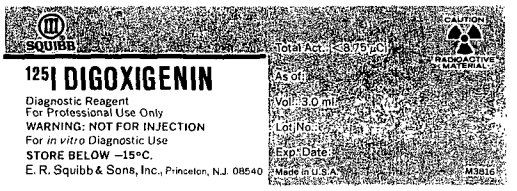
CONTENTS: 1 Vial—^{125}I DIGOXIGENIN
1 Vial (2.5 ml.) — DIGOXIN STANDARD
1 Vial (5.0 ml.) — DIGOXIN ANTISERUM
1 Vial (1.2 g.) — BARBITAL BUFFER MIXTURE
1 Vial (0.75 g.) — POWDERED CHARCOAL
1 Vial (0.5 g.) — BOVINE SERUM ALBUMIN POWDER
1 Vial (2.42 g.) — TRIS(HYDROXYMETHYL)AMINOMETHANE (TRIS)

STORE BELOW — 15°C. See enclosed directions

EXP. DATE: **LOT NO.:**

E. R. Squibb & Sons, Inc., Princeton, N. J. 08540 Made in U.S.A. M5278A

59649



^{125}I DIGOXIGENIN

Diagnostic Reagent
For Professional Use Only
WARNING: NOT FOR INJECTION
For *in vitro* Diagnostic Use
STORE BELOW –15°C.
E. R. Squibb & Sons, Inc., Princeton, N.J. 08540

DIGOXIN IMMUTOPE® Kit

List 09360

Diagnostic Reagent • For Professional Use Only
For *in vitro* Diagnostic Use • WARNING: NOT FOR INJECTION
Contains sufficient material for 200 radioimmunoassay tests
Not for Internal or External Use in Humans or Animals

CONTENTS: 1 Vial-^{125}I DIGOXIGENIN
1 Vial (2.5 ml.) — DIGOXIN STANDARD
1 Vial (10 ml.) — DIGOXIN ANTISERUM
1 Vial (2.4 g.) — BARBITAL BUFFER MIXTURE
1 Vial (1.5 g.) — POWDERED CHARCOAL
1 Vial (1 g.) — BOVINE SERUM ALBUMIN POWDER
1 Vial (4.84 g.) — TRIS (HYDROXYMETHYL) AMINOMETHANE (TRIS)

STORE BELOW – 15°C. **See enclosed directions**

EXP. DATE: **LOT NO.:**

E. R. Squibb & Sons, Inc., Princeton, N. J. 08540 Made in U.S.A. M5335

SQUIBB

Digoxin IMMUTOPE® Kit

For Quantitative Measurement of Serum or Plasma Digoxin Levels by Radioimmunoassay

For *IN VITRO* Diagnostic Use
For Professional Use Only

DETERMINATION OF SERUM OR PLASMA 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. When equilibrium has been reached in the antigen-antibody reaction, the free and bound components of the mixture are separated, and the relative amounts of each are determined by measuring the radioactivity of the separated components. 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 IMMUTOPE Kit, antibody to digoxin (raised in rabbits by administration of digoxin coupled to human serum albumin) serves as the specific antibody, purified digoxigenin labeled with ^{125}I serves as the labeled antigen, and purified digoxin is used as a reference standard. Powdered charcoal is used to separate free digoxin from antibody-bound digoxin, and digoxin levels are expressed as nanograms (ng., 10^{-9}g.) of digoxin per milliliter of serum or plasma (ng./ml.).

RATIONALE FOR USE

The measurement of serum or plasma levels of digoxin by radioimmunoassay has proved to be a valuable adjunct in the clinical diagnosis of digoxin toxicity. Since excessive accumulation of digoxin is a major factor in the development of toxicity, and there is some constancy in myocardium-to-serum digoxin ratios,[1] determination of serum digoxin concentration can be of help in the diagnosis of digoxin intoxication.[2]

Measurement of serum or plasma digoxin by radioimmunoassay has the advantages of speed and simplicity over the originally used double-isotope derivative method[3] and has the advantages of simplicity and specificity over bioassay methods.[4] The more recently developed ^{86}Rb uptake method has yielded results that are in good agreement with results obtained by radioimmunoassay, but requires a day to obtain results versus one hour required for radioimmunoassay.[5]

The Digoxin IMMUTOPE Kit utilizes ^{125}I labeled digoxigenin in lieu of ^{3}H labeled digoxin that is employed in many of the procedures described in the literature. The use of ^{125}I rather than ^{3}H avoids the problems associated with sample preparation and availability of liquid scintillation counting equipment. In addition, the use of internal counting standards to correct for quenching associated with the presence of variable quantities of bile pigments or hemoglobin[6] is avoided with the ^{125}I label. The half-life of ^{125}I is 60 days. The isotope decays in a complex fashion with emission of x-rays and gamma rays whose radiation energies are 27.5 kev and 35.4 kev, respectively. These energies are well within the detection capability of modern solid crystal gamma scintillation detectors. There is no beta emission.

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 IMMUTOPE Kit.

Biological Properties: Digoxin is well absorbed from the gastrointestinal tract, with approximately 80 percent of an oral dose being eventually absorbed. Following oral administration, peak serum levels are found at one to two hours. Absorption is not diminished by food or fasting, although the shape of the curve defined by serum levels has a somewhat lower and more extended peak.[7]

Doherty[8] and co-workers administered tritium labeled digoxin to human subjects and found a more or less constant relationship between tissue and serum levels. Concentrations of digoxin were always highest in the heart, followed by liver and kidney. The ratio between heart and serum concentration was rather constant at 30:1.[8] The relationship between tissue and serum concentration and the fairly uniform serum concentration over several hours during the post-absorptive phase, provides the basis for the clinical use of serum digoxin determinations.[9]

There is some controversy as to the nature of the binding (specific vs. nonspecific), but it is generally accepted that digoxin forms a complex with the plasma membrane-bound enzyme, Na^+, K^+ ATP-ase. It has been shown that all cardiac glycosides inhibit ATP-ase activity in cardiac and other tissues, and that concentrations causing inhibition are in a range known to cause a positive inotropic effect.[7] As with other cardiac glycosides, digoxin acts primarily on the heart to (1) increase the force of systolic contraction; (2) slow conduction and lengthen the refractory period through the AV node and bundle of His; and (3) alter cardiac vagal activity.

Digoxin is excreted largely unchanged in the urine. Loss in the stool accounts for about 15 percent of a single dose, with virtually all of this being derived from bile. Of clinical importance is the direct relationship between glomerular filtration rate and the clearance of digoxin. Patients with renal impairment have a significantly prolonged serum half-life and tend to accumulate the drug. Anephric subjects exhibit a very long serum half-life and have markedly increased excretion via the stool, apparently representing a secondary adaptive mechanism for limiting the progressive accumulation of digoxin in such patients.[7]

CLINICAL APPLICATIONS

The clinical use of digitalis and its component glycosides is accompanied by a distressingly high prevalence of toxic manifestations, the most serious of which are arrhythmias and disturbance of conduction. Many factors contribute to the development of digitalis toxicity, with perhaps the most important factor being an accumulation of excessive amounts of digitalis in the body and in the myocardium in particular.[10] Since there is a degree of correlation between serum and tissue levels of digoxin, the measurement of serum digoxin levels provides useful information in the diagnosis of digoxin toxicity. In view of the multiple factors governing individual response to cardiac glycosides, however, it must be stressed that serum digoxin measurements should be viewed as just one of many important factors to be weighed in a complex clinical setting.[11]

The measurement of serum digoxin levels, in conjunction with careful investigation of adherence to prescribed medication schedules, has also been of value in detecting patients who fail to comply with the prescribed dosage regimen.[12,13]

J3-313B

CLINICAL STUDIES

Studies from a number of laboratories, using several different techniques, reflect substantial agreement concerning serum or plasma digoxin levels in patients receiving usual doses of this drug. Beller et al.[14] conducted a prospective study of 931 consecutive patients admitted to a medical service at their institution, to describe the prevalence and epidemiology of cardiac digitalis toxicity, and to correlate serum concentrations of digoxin (measured by radioimmunoassay) with clinical and biochemical data. Fifteen percent of the patients surveyed were taking digitalis on admission, and of these, 23 percent were definitely toxic, and 6 percent possibly toxic, as determined by serial electrocardiograms. There was a significantly greater prevalence of advanced heart disease, underlying atrial fibrillation, anorexia, acute or chronic pulmonary disease, and renal failure in toxic patients, versus nontoxic patients. Mortality was more than twice as high in the toxic group versus the nontoxic group. The mean serum digoxin concentration in toxic patients was 2.3 ± 1.6 (±S.D.) ng./ml., whereas the mean level in the nontoxic group was 1.0 ± 0.5 ng./ml. ($p<0.005$). The degree of overlap between the two groups was somewhat greater than reported by other investigators, and was attributed to the prospective nature of the study and the long time interval (i.e., up to 48 hours) between the last dose of digitalis and the performance of the serum digoxin assay. It was emphasized that because of the overlap of serum digitalis levels in clinically toxic and nontoxic patients, sole reliance on these levels for the determination of the presence or absence of digitalis toxicity is not warranted. Knowledge of serum glycoside levels was found most useful when weighed in the entire clinical context.

In a previous study, Smith and Haber[11] reported a mean serum digoxin level of 3.7 ± 1.0 (S.D.) ng./ml. for toxic patients, and a mean of 1.4 ± 0.7 ng./ml. for nontoxic patients. Ninety percent of patients without evidence of toxicity had serum digoxin concentrations of 2.0 ng./ml. or less, while 87 percent of the toxic group had levels above 2.0 ng./ml. Blood samples from these patients were obtained between 8 and 12 hours after the last dose of digoxin.

Johnston et al.[15] reported mean plasma digoxin levels of 0.92 ± 0.19 ng./ml. for adequately digitalized patients taking 0.25 mg. of digoxin per day, while patients taking 0.5 mg. per day had a mean plasma level of 1.23 ± 0.1 ng./ml. Twenty-one patients who fulfilled the criteria for digitalis toxicity had mean digoxin levels of 3.15 ± 0.25 ng./ml. for the 0.25 mg./day dosage, and 3.10 ± 1.18 ng./ml. for the 0.5 mg./day dosage. All but three of the toxic patients showed some degree of renal insufficiency. In this study, the blood was drawn for assay three hours after the last digitalis dose.

Zeegers et al.[16] recently reported on a group of 93 well digitalized patients and 22 patients considered toxic. The mean digoxin level for the nontoxic patients was 1.6 ± 0.7 ng./ml. (range 0.4 – 3.5 ng./ml.), while the toxic group had a mean plasma level of 4.4 ± 0.9 ng./ml. It was concluded that the difference between the mean digoxin levels in toxic and nontoxic patients was significant, however, a certain degree of overlapping did exist. It was also concluded that the blood sample should be withdrawn at least six hours after the last dose of digoxin to correctly judge the plasma digoxin level.

Park et al.[2] measured serum digoxin levels in a group of 129 adult patients, and found a mean digoxin level of 1.1 ± 0.1 (1 S.E.) ng./ml. for 108 nontoxic patients, and a mean level of 3.8 ± 0.5 ng./ml. for 21 toxic patients. An overall accuracy of 89 percent was reported for the method in differentiating between toxic and nontoxic patients. Ninety percent of the nontoxic patients had serum digoxin levels below 2 ng./ml., while 86 percent of the toxic patients had levels above 2 ng./ml. Blood samples were drawn between 5 and 30 hours after the last digoxin dose.

In a review article, Smith and Haber[6] observed that despite the multiple variables known to influence cardiac response to digitalis glycosides, significantly higher mean serum digoxin levels were observed in toxic patients compared with nontoxic patients in nearly all studies published to date; the study of Fogelman et al.[17] being the only exception. Results on over 1,000 patients have shown that mean digoxin levels in patients with toxic manifestations are about two-fold higher than those of patients without toxicity. They point out that despite the significantly different mean levels, however, overlap has been observed in most series, and it must be emphasized that no arbitrary level can be chosen which clearly differentiates toxic from nontoxic serum digoxin concentrations. They conclude that serum digoxin levels are most useful when interpreted together with all other relevant variables in the clinical context.

REAGENTS

The Digoxin IMMUTOPE Kit is available in 100-test and 200-test packages. The 100-test kit contains one vial of ^{125}I Digoxigenin (3.0 ml., with a total activity of <8.75 μCi), one vial of Digoxin Standard (2.5 ml.), one vial of Digoxin Antiserum (5.0 ml.), one vial of Barbital Buffer Mixture (containing 0.18 g. sodium barbital, 0.92 g. sodium chloride, 0.12 g. sodium acetate trihydrate, for a total of 1.2 g.), one vial of Powdered Charcoal (0.75 g.), one vial of Bovine Serum Albumin Powder (0.5 g.), and one vial of Tris(hydroxymethyl)aminomethane (TRIS) (2.42 g.).

The 200-test kit contains one vial of ^{125}I Digoxigenin (3.0 ml., with a total activity of <8.75 μCi), one vial of Digoxin Standard (2.5 ml.), one vial of Digoxin Antiserum (10.0 ml.), one vial of Barbital Buffer Mixture (containing 0.35 g. sodium barbital, 1.84 g. sodium chloride, 0.23 g. sodium acetate trihydrate, for a total of 2.4 g.), one vial of Powdered Charcoal (1.5 g.), one vial of Bovine Serum Albumin Powder (1.0 g.), and one vial of Tris(hydroxymethyl)aminomethane (TRIS) (4.84 g.).

WARNINGS

For *In Vitro* Diagnostic Use. For Professional Use Only.

Note: 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 general license of the U.S. Atomic Energy 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 Digoxin IMMUTOPE Kit requires only a general license from the Atomic Energy Commission. The general license is issued to any physician, clinical laboratory, or hospital who obtains a validated registered A.E.C. Form 483. This form must be submitted in triplicate to the A.E.C. 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 A.E.C. for quantities larger than 200 microcuries).

PRECAUTIONS

The by-product material should be stored in the original shipping container or in a container providing equivalent radiation protection until used. Observe the following precautions in handling radioactive material: 1) There should be no pipetting by mouth, 2) There should be no smoking or eating while radioactive materials are being handled, 3) Cover hands with rubber gloves during and wash thoroughly after handling radioactive materials. 4) Wipe up spills quickly and thoroughly; add the contaminated materials to radioactive waste matter. 5) Solid waste can be stored until it decays. It may be discarded in the customary manner, after removing labeling. When the radioactive material used has a relatively short physical half-life, contaminated material may be stored in a specifically designated area in a covered metal or plastic container conspicuously marked with a radiation caution label. Monitor each piece of material for radioactivity and store until contamination no longer persists. Radioactive material with a relatively long physical half-life may best be removed by special disposal services available throughout the country. For a complete description of proper waste disposal, reference should be made to Title 10, Code of Federal Regulations, Part 20.

PREPARATION OF TEST REAGENTS—100-TEST PACKAGE

1. ***Tris Acetate Buffer with BSA:*** Transfer the entire contents (2.42 g.) of the vial containing Tris(hydroxymethyl)aminomethane (TRIS) and the entire contents (0.5 g.) of the vial containing bovine serum albumin powder to a suitable container and add 200 ml. of distilled water. After dissolving the solids, adjust the solution to pH 7.4 ± 0.5 using a few drops (~1 ml.) of glacial acetic acid. **Store final solution at 2–8° C.**
2. ***Charcoal Suspension:*** Transfer the entire contents of the vial containing the Barbital Buffer Mixture (1.2 g.) to a suitable container and add 120 ml. of distilled water. Adjust the solution to pH 7.4 ± 0.5 by adding 0.2N HCl (~3.4 ml.). The 0.2N HCl can be prepared by diluting 2 ml. of concentrated HCl to 125 ml. with distilled water.

   To prepare the charcoal suspension, add the entire contents (0.75 g.) of the vial containing powdered charcoal to the above solution and stir vigorously for several minutes. ***Caution should be used in opening the charcoal vial, especially if opened immediately after removing from storage at freezing temperature. To avoid forceful expulsion of the contents of the vial, it should be vented with a hypodermic needle prior to removing the aluminum seal.*** **Store the suspension at 2–8° C. and stir vigorously before removing aliquots for use.**
3. ***Diluted ^{125}I Digoxigenin:*** Dilute a portion of the ^{125}I Digoxigenin supplied with the kit with the Tris Acetate Buffer with BSA (prepared in Step #1 above), so that the final solution has a radioactivity concentration of approximately 5,000 counts per minute per milliliter. Approximately 0.2 ml. of the radioactive solution diluted to 100 ml. will give the desired ~5,000 cpm/ml. The required volume will vary with the efficiency and discriminator settings on counting equipment used. **The diluted ^{125}I Digoxigenin should be stored at 2–8° C. and prepared fresh each week.**

PREPARATION OF TEST REAGENTS—200-TEST PACKAGE

1. ***Tris Acetate Buffer with BSA:*** Transfer the entire contents (4.84 g.) of the vial containing Tris(hydroxymethyl)aminomethane (TRIS) and the entire contents (1.0 g.) of the vial containing bovine serum albumin powder to a suitable container and add 400 ml. of distilled water. After dissolving the solids, adjust the solution to pH 7.4 ± 0.5 by adding glacial acetic acid (~2 ml.). **Store final solution at 2–8° C.**
2. ***Charcoal Suspension:*** Transfer the entire contents of the vial containing the Barbital Buffer Mixture (2.4 g.) to a suitable container and add 240 ml. of distilled water. Adjust the solution to pH 7.4 ± 0.5 by adding 0.2N HCl (~6.8 ml.). The 0.2N HCl can be prepared by diluting 3 ml. of concentrated HCl in 125 ml. of distilled water.

To prepare the charcoal suspension, add the entire contents (1.5 g.) of the vial containing powdered charcoal to the above solution and stir vigorously for several minutes. ***Caution should be used in opening the charcoal vial, especially if opened immediately after removing from storage at freezing temperature. To avoid forceful expulsion of the contents of the vial, it should be vented with a hypodermic needle prior to removing the aluminum seal.*** **Store the suspension at 2–8° C. and stir vigorously before removing aliquots for use.**

3. *Diluted ^{125}I Digoxigenin:* Prepare this solution in exactly the same manner as described in Step #3 under Preparation of Test Reagents—100-Test Package.

NOTE: With the exception of the diluted ^{125}I Digoxigenin, all reagents supplied with the kits, and the reagents prepared above, will remain stable for the useful life of the kit with which they were supplied, provided recommended storage conditions are adhered to. Unopened kits should be stored below −15° C.

COLLECTION OF BLOOD SAMPLES FOR ANALYSIS

An appropriate quantity of blood should be collected from a peripheral vein using a standard blood collection tube. The requirement for an anticoagulant in the tube will depend on whether the test will be conducted on serum or plasma. Blood samples that are not processed on the day they are collected, should be stored under refrigeration. Serum or plasma samples should be frozen if not assayed within 48 hours of sample collection.[18]

There is considerable variability in the literature on the time at which the blood sample should be drawn following the last dose of digoxin, with intervals ranging from 3 to 48 hours being reported. Redfors[19] claims that an interval of 24 hours provided a steady state condition that gave the most reliable reflection of the digoxin concentration in the myocardium, and gave good day to day reproducibility. Most other investigators used intervals of 4 to 12 hours, with the general consensus being that an interval of at least six hours should be allowed for serum or plasma concentrations to reach a plateau. Regardless of the time interval used, it is strongly recommended that the time interval be recorded for each specimen drawn, and the digoxin level be expressed in ng./ml. for the chosen time interval. It is self-evident that the greatest diagnostic accuracy will be attained by establishing toxic and nontoxic levels at a specific time interval. The same levels and time intervals should be used for subsequent diagnostic studies.

In addition to specifying the time interval between blood collection and the last digoxin dose, the patient history should be thoroughly scrutinized to determine if any diagnostic or therapeutic radioisotopes have been administered to the patient in the week or two immediately prior to the digoxin assay. It is also important for the assay request to specify the exact drug being used (i.e., digoxin, digitoxin, or digitalis), to avoid erroneous assay results.

There is no requirement that the patient be in the fasting state at the time of administration of the last digoxin dose or during the interval prior to collection of the blood sample.

RADIOIMMUNOASSAY TEST PROCEDURE

Materials Needed

In addition to the reagents and materials supplied with the Digoxin IMMUTOPE Kits, the following equipment is required:

1. Centrifuge capable of 3000-4000 rpm
2. Well-type gamma scintillation counter
3. pH meter and standards
4. Refrigerator
5. Freezer
6. Magnetic stirrer and stirring bars
7. Test tubes, Squibb Standard Test Tube, List #09020
8. Glacial acetic acid
9. 0.2N hydrochloric acid
10. Oxford sampler and tips for 1.0 ml. and 100 μl. (or equivalent)
11. Hamilton repeating syringes and holders, 0.5 ml. to deliver 10 μl., and 2.5 ml. to deliver 50 μl. (or equivalent)
12. Container for radioactive waste
13. Usual bench equipment including racks, graduates, and beakers of appropriate sizes to accommodate the quantities of reagents used in this test.

Important

It will take approximately one hour for the frozen reagents to thaw upon removal from the styrofoam platform. The ^{125}I Digoxigenin solution is not expected to solidify at the recommended storage temperature.

Repeated thawing and refreezing of the Digoxin Standard and Digoxin Antiserum do not significantly affect test results. It is recommended that aliquots be removed from the original containers by means of a hypodermic needle and syringe. **The Digoxin Standard and Antiserum should be stored in their original containers, with their original rubber closures. Do not transfer these reagents to another container or change the rubber closure supplied in the vials.**

Smaller volumes of serum or plasma may be used in the radioimmunoassay test procedure if high digoxin levels are anticipated. The calculation of digoxin levels should be modified accordingly to reflect the aliquot used in the test.

During the separation step, the charcoal suspension should be added only to the quantity of tubes that can be centrifuged simultaneously. The equilibrium of the antigen-antibody reaction is altered upon prolonged contact with the charcoal. After addition of the charcoal suspension, samples should be centrifuged as quickly as possible.

To facilitate accuracy in the transfer of the supernatant to its counting tube, it is recommended that the rims of the two tubes be touched to effect complete transfer of the liquid.

Reagents from individual kits should not be intermixed with reagents from other kits.

Procedure

The procedure described below is based on performance of duplicate analyses on all samples assayed. Read entire procedure before starting test.

1. Mark a series of test tubes with the numbers 1 through 14. The first 12 tubes are required for preparation of the standard curve and the remaining two tubes are required for assay of one clinical sample. Two additional tubes should be used for each additional clinical sample to be assayed.
2. Add 1 ml. of diluted ^{125}I Digoxigenin to each tube.
3. Add the following quantities of Digoxin Standard to the respective tubes: tubes #3 and #4–5 μl.; tubes #5 and #6–10 μl; tubes #7 and #8–20 μl.; tubes #9 and #10–30 μl.; tubes #11 and #12–50 μl.
4. Add 100 μl. of the clinical sample to tubes #13 and #14. The composition of the 14 tubes is summarized below:

Tube #	Sample Added	Digoxin Content ($\mu\mu$g.)
1 and 2	None	0
3 and 4	D Std (5 μl.)	50
5 and 6	D Std (10 μl.)	100
7 and 8	D Std (20 μl.)	200
9 and 10	D Std (30 μl.)	300
11 and 12	D Std (50 μl.)	500
13 and 14	Clin. Sample (100 μl.)	unknown

5. Add 50 μl. of Digoxin Antiserum to all 14 tubes, mix gently, and incubate at room temperature (22°–24° C.) for 30 minutes.
6. At the end of the incubation period add 1 ml. of the charcoal suspension to all tubes. The suspension should be kept under constant agitation during removal of the 1 ml. aliquots.
7. Mix the sample gently, and then centrifuge for 2 - 3 minutes at 3000 - 4000 rpm.
8. Decant the supernatant from each tube into correspondingly numbered tubes.
9. Measure the radioactivity in all tubes (i.e., tubes containing charcoal residue and tubes containing supernatant) by counting in a standard well-type gamma scintillation counter (discriminator settings 20 - 50 kev). Subtract background cpm and record net cpm for each tube on the worksheet provided with the kit.
10. Calculate the % Bound (digoxin bound to antibody) for each of the original tubes using the following formula, and record results on the worksheet:

$$\% \text{ Bound} = \frac{\text{Activity in Supernatant tube (cpm)} \times 100}{\text{Activity in Supernatant tube (cpm)} + \text{Activity in charcoal tube (cpm)}}$$

For example: Tube #1 Supernatant - 2400 cpm
Tube #1 Charcoal - 2600 cpm

$$\% \text{ Bound (Tube \#1)} = \frac{2400 \times 100}{2400 + 2600} = 48\%$$

11. Calculate the average % Bound values for the duplicate samples and record average values on worksheet.
12. Prepare a Standard Curve by plotting the average % Bound values against the quantity of Digoxin Standard added to the respective tubes (i.e., tubes 1 through 12). See the sample standard curve depicted on the worksheet for guidance in plotting the data. The standard curve shown on the worksheet is provided for guidance only, and **should not** be used in calculating digoxin levels in clinical samples.
13. Determine the quantity ($\mu\mu$g.) of digoxin in the clinical sample by referring to the Standard Curve prepared above. The % Bound value for each sample will correspond to the specific quantity of digoxin contained in the sample.
14. Calculate the patient serum or plasma digoxin level as follows:

$$\text{Serum or plasma Digoxin (ng./ml.)} = \frac{\mu\mu\text{g. Digoxin in Sample} \times 10}{1000}$$

For example:
Clinical sample contains 150 $\mu\mu$g. Digoxin

$$\text{Serum or Plasma Digoxin} = \frac{150 \times 10}{1000} = 1.5 \text{ ng./ml.}$$

PARAMETERS OF THE TEST PROCEDURE

The specificity and sensitivity of the test procedure depends on the specific characteristics of the antiserum provided in the individual kit shipments. In general terms, antiserum produced in a manner similar to that supplied in the Digoxin IMMUTOPE Kit has shown some cross-reactivity with digitoxin, while other steroid compounds, less similar to digoxin than digitoxin, were essentially without effect in the assay system.[20]

The sensitivity of the assay system is a function of the avidity of the specific antiserum employed in the assay system. The sensitivity of the Digoxin IMMUTOPE Kit is at least as great as the 0.1 ng./ml. sensitivity reported by Smith and Haber.[6]

The reproducibility of test results is based on a great number of variables, not the least of which is the attention to detail exercised by the individual performing the test. Exercising a reasonable degree of care, reproducibility of Digoxin IMMUTOPE Kit results should be well within the 7 percent standard deviation for replicate assays, and the 12 percent standard deviation for assays conducted on the same samples on different days, reported in the literature for a similar assay system.[20]

Studies on the recovery from serum of added digoxin, have been conducted with the Digoxin IMMUTOPE Kit. Recoveries were within 10 percent of the expected digoxin values using serum samples of both 50 μl. and 100 μl.[21]

Studies have been conducted with the Digoxin IMMUTOPE Kit in several laboratories to establish the results obtained with the kit.

Serum digoxin levels were determined for 196 patients (235 determinations) adequately digitalized. The average value for these determinations was 0.98 ng./ml. for serum analyzed four to six hours after the last dose of digoxin. The range of values for these patients was 0.0-3.2 ng./ml., with 80 percent of the values falling between 0.5 and 1.99 ng./ml.

Serum digoxin determinations were made as above from 36 patients with digoxin toxicity. The digoxin levels for 31 of these patients averaged 3.05 ng./ml., with a range of 2.1 to 7.4 ng./ml. Five of these patients had digoxin levels between 0.06 and 1.1 ng./ml.

Twenty patients considered to have possible digoxin toxicity were shown to have serum values ranging between 0.2 and 6.0 ng./ml. Sixty percent (60%) of these had values of 2.0 ng./ml. or higher.

The overlap between serum digoxin levels for digoxin toxic and digitalized but nontoxic patients had been noted by laboratories evaluating patients with other methods for serum digoxin concentrations.

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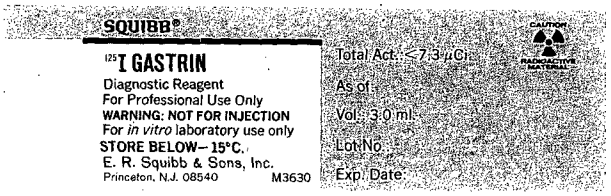
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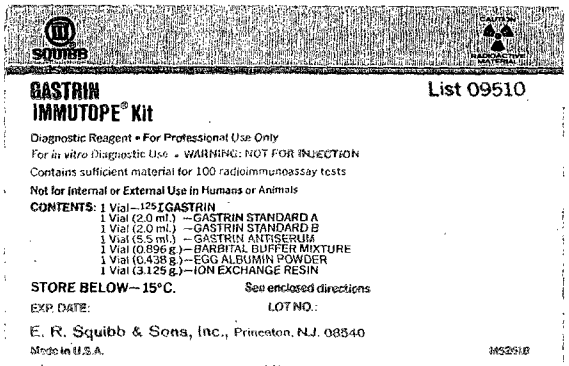
M3630
Total Act.: <7.3 μCi
As of
Vol.: 3.0 ml
Lot No.
Exp. Date:
CAUTION
RADIOACTIVE MATERIAL


SQUIBB
CAUTION
RADIOACTIVE MATERIAL
GASTRIN
IMMUTOPE® Kit
List 09510
Diagnostic Reagent • For Professional Use Only
For *in vitro* Diagnostic Use • WARNING: NOT FOR INJECTION
Contains sufficient material for 100 radioimmunoassay tests
Not for Internal or External Use in Humans or Animals
CONTENTS: 1 Vial—^{125}I GASTRIN
1 Vial (2.0 mL) —GASTRIN STANDARD A
1 Vial (2.0 mL) —GASTRIN STANDARD B
1 Vial (5.5 mL) —GASTRIN ANTISERUM
1 Vial (0.896 g)—BARBITAL BUFFER MIXTURE
1 Vial (0.438 g)—EGG ALBUMIN POWDER
1 Vial (3.125 g)—ION EXCHANGE RESIN
STORE BELOW—15°C.
See enclosed directions
EXP. DATE:
LOT NO.:
E. R. Squibb & Sons, Inc., Princeton, N.J. 08540
Made in U.S.A.
IMS2510

SQUIBB

Gastrin IMMUTOPE® KIT

For Quantitative Measurement of Serum Gastrin Levels by Radioimmunoassay

For *In Vitro* Diagnostic Use

For Professional Use Only

DETERMINATION OF SERUM GASTRIN LEVELS BY RADIOIMMUNOASSAY

Measurement of body constituents 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. When equilibrium has been reached in the antigen-antibody reaction, the free and bound components of the mixture are separated, and the relative amounts of each are determined by measuring the radioactivity of the separated components. 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 Gastrin IMMUTOPE Kit, antibody to synthetic human gastrin I serves as the specific antibody, synthetic human gastrin I labeled with iodine-125 serves as the labeled antigen, and synthetic human gastrin I is used as a reference standard. An anion exchange resin is used to separate free gastrin from antibody-bound gastrin, and serum gastrin levels are expressed as picograms (pg., 10^{-12} g.) of gastrin per milliliter of serum (pg./ml.).

RATIONALE FOR USE

The measurement of serum gastrin levels by radioimmunoassay has been of value in studying physiological processes involving the gastrointestinal hormones,[1-5] and as an aid in detecting tumors of the Zollinger-Ellison variety.[2,6-8] The measurement of serum gastrin levels by radioimmunoassay has several advantages over the standard bioassay procedures. The sensitivity, simplicity, specificity, and rapidity with which the radioimmunoassay can be performed, remove the obstacles to routine clinical determination of serum gastrin that were associated with the more complex bioassay procedure.

The use of ^{125}I rather than ^{131}I considerably lengthens the shelf-life of the radioiodinated Gastrin employed in the test, and reduces radiation exposure to laboratory personnel. The half-life of ^{125}I is 60 days. The isotope decays in a complex fashion with emission of x-rays and gamma rays whose radiation energies are 27.5 kev and 35.4 kev, respectively. These energies are well within the detection capability of modern counting equipment. There is no beta emission.

GASTRIN CHEMICAL AND BIOLOGICAL PROPERTIES

Gastrin is a linear polypeptide with seventeen amino acid residues (heptadecapeptide), that is produced by the mucosal lining of the gastric antrum.[9] Gastrin release is stimulated by feeding, alkaline pH, cholinergic stimulation and mechanical distention of the gastric antrum. Human gastrin occurs in two almost identical chemical forms, which possess identical biological activities. Human gastrin I is a heptadecapeptide with a molecular weight of 2,096; human gastrin II is a heptadecapeptide with a molecular weight of 2,176, due to the presence of a sulfate ester on the tyrosyl residue at position 12. Both terminal groups of the gastrin molecule are blocked: the N-terminal by the formation of the pyroglutamyl condensation, and the C-terminal by the presence of an amide. The excess of dicarboxylic acids in gastrin confers a strong negative charge to the molecule, which is reflected in its electrophoretic and chromatographic behavior.[10] Gastrin is adsorbed by anion exchange resins.

Yalow and Berson[11] have studied the nature of immunoreactive gastrin in human plasma and extracts from gastrointestinal tissues, and have observed the presence of two immunoreactive components of plasma gastrin. One component has the characteristics of heptadecapeptide gastrin, while the other component (BG, big gastrin) has a less acidic charge than heptadecapeptide gastrin and based on Sephadex gel column chromatography has an estimated molecular weight of about 7000. The BG component usually represents the major fraction of plasma gastrin, and appears to have the same biologic and immunologic potencies as heptadecapeptide gastrin.

The primary biologic response to gastrin is the production of hydrochloric acid by the parietal cells of the stomach. Release of gastrin from the antral mucosa into the circulation, following a variety of stimuli, results in a potent stimulus to gastric secretion of hydrochloric acid. Gastrin release is inhibited when the intragastric pH is reduced to 3.0 and eliminated at pH 1.5 or less,[2] completing a cycle of feed back controls.

Gastrin has effects on all major gastrointestinal activities including secretion, motility, and absorption. These physiological and pharmacological actions are discussed in detail in papers by Sanders and Schimmel,[9] and Grossman.[10]

CLINICAL APPLICATIONS

By use of the radioimmunoassay technique, fasting gastrin levels have been shown to be markedly elevated in patients

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with Zollinger-Ellison (ZE) syndrome. This disease is caused by a nonbeta pancreatic islet cell tumor producing excessive amounts of gastrin, and is characterized by high basal acid secretion and intractable peptic ulceration. More conventional varieties of peptic-ulcer disease are often, but not always, associated with increased rates of gastric acid secretion, but unlike patients with ulcer disease of the Zollinger-Ellison type, fasting serum gastrin levels have not been elevated.

Serum gastrin levels in the fasting and the postprandial state are also markedly increased in patients with pernicious anemia, and in many cases, the levels are sufficiently elevated to fall in the range characteristic of patients with ZE syndrome. In pernicious anemia patients, elevated gastrin levels, which are apparently due to high intragastric pH, are promptly and substantially reduced after ingestion of hydrochloric acid.

CLINICAL STUDIES

Normal Values: Trudeau and McGuigan[2] reported fasting serum gastrin levels of 85 ± 9.8 pg./ml. in 35 normal volunteers. Berson and Yalow[7] reported fasting plasma gastrin levels in normal adult men that averaged 30 pg./ml. with a median of 15 pg./ml., attributing the lower values to statistical analysis of the clinical results and differences in age and basal acid secretion rates for the groups studied. Diminished immunochemical potency of the gastrin standard used in the assay procedures yielding higher normal values was also cited as possibly contributing to the differences in absolute values reported by different laboratories.

Zollinger-Ellison Syndrome: Berson and Yalow[7] have reported basal plasma gastrin levels in the ZE syndrome ranging from 300 pg./ml. to 10 ng./ml., with occasional values as high as 300 ng./ml. To further differentiate between normal subjects and patients with Zollinger-Ellison syndrome, the authors reported a three-to-four-fold increase in plasma gastrin in normal subjects following a test meal (4 oz. orange juice, two eggs, and one piece of dry toast), while ZE patients showed no rise in plasma gastrin levels after feeding. Conversely, the infusion of calcium causes only a slight increase in serum gastrin levels in normal subjects, but may cause a very large increase in patients with ZE syndrome.[12]

Pernicious Anemia: Patients with pernicious anemia (PA) are characterized by atrophy of the gastric mucosa and absence of gastric acid secretion and intrinsic factor. McGuigan and Trudeau[13] have reported mean fasting serum gastrin levels of 997 (±182 S.E.) pg./ml. in 20 patients with pernicious anemia, with nine of the patients having serum gastrin levels sufficiently elevated to be considered in the hypergastrinemic range characteristic of the ZE syndrome. An abrupt fall in plasma gastrin concentration toward normal was observed by Yalow and Berson[6] following instillation of 300 ml. of 0.1N hydrochloric acid to five patients with pernicious anemia.

REAGENTS

The Gastrin IMMUTOPE Kit is available in a 100-test package. The kit contains one vial of ^{125}I Gastrin (3.0 ml. with a total activity of <7.3 μCi), one vial of Gastrin Standard A (2.0 ml.), one vial of Gastrin Standard B (2.0 ml.), one vial of Gastrin Antiserum (5.5 ml.), one vial of Barbital Buffer Mixture (containing 0.760 g. sodium barbital and 0.136 g. barbital, for a total of 0.896 g.) one vial of Egg Albumin Powder (0.438 g.) and one vial Ion Exchange Resin (3.125 g.).

WARNINGS

For *In Vitro* Diagnostic Use. For Professional Use Only.

Note: 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 general license of the U.S. Atomic Energy Commission or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority.

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In vitro clinical laboratory testing with the Gastrin IMMUTOPE Kit requires only a general license from the Atomic Energy Commission. The general license is issued to any physician, clinical laboratory, or hospital who obtains a validated registered A.E.C. Form 483. This form must be submitted in triplicate to the A.E.C. 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 A.E.C. for quantities larger than 200 microcuries.)

PRECAUTIONS

The by-product material should be stored in the original shipping container or in a container providing equivalent radiation protection until used. Observe the following precautions in handling radioactive material: 1) There should be no pipetting by mouth. 2) There should be no smoking or eating while radioactive materials are being handled. 3) Cover hands with rubber gloves during and wash thoroughly after handling radioactive materials. 4) Wipe up spills quickly and thoroughly; add the contaminated materials to radioactive waste matter. 5) Solid waste can be stored until it decays. It may then be discarded in the customary manner, after removing labeling. When the radioactive material used has a relatively short physical half-life, contaminated material may be stored in a specifically designated area in a covered metal or plastic container conspicuously marked with a radiation caution label. Monitor each piece of material for radioactivity and store until contamination no longer persists. Radioactive material with a relatively long physical half-life may best be removed by special disposal services available throughout the country. For a complete description of proper waste disposal, reference should be made to Title 10, Code of Federal Regulations, Part 20.

PREPARATION OF TEST REAGENTS

1. *Barbital Buffer Solution:* Transfer the entire contents (0.896 g.) of the vial containing the Barbital Buffer Mixture to a suitable container and add 200 ml. of distilled water. Stir until solution is complete. The resulting solution is to be used for preparation of the reagents listed below.
2. *Ion Exchange Resin Suspension:* Transfer the entire contents (3.125 g.) of the vial containing the Ion Exchange Resin to a suitable container and add 25 ml. of the Barbital Buffer Solution prepared in Step #1 above. **Store the suspension at 2°-8°C. and stir vigorously during removal of aliquots for use in the separation step.**
3. *Barbital Buffer with Egg Albumin:* Add the entire contents (0.438 g.) of the vial containing the Egg Albumin Powder to the remaining 175 ml. of Barbital Buffer Solution prepared in Step #1 above. Stir until solution is complete. **Store at 2°-8°C.**
4. *Diluted ^{125}I Gastrin Solution:* Dilute a portion of the ^{125}I Gastrin supplied with the kit with the Barbital Buffer with Egg Albumin (prepared in Step #3 above), so that the final solution has a radioactivity concentration of approximately 4000 counts per minute per milliliter. Approximately 0.2 ml. of the radioactive solution diluted to 100 ml. will give the desired ~4000 cpm/ml. The required volume will vary with the efficiency and discriminator settings on the counting equipment used. Only that quantity of diluted ^{125}I Gastrin required for the number of assays performed in a one-week period should be prepared at any given time. **The diluted ^{125}I Gastrin should be stored at 2°-8°C. and prepared fresh each week. To avoid forceful expulsion of the contents of the vial, it should be vented through the rubber stop-**

per with a hypodermic needle prior to removing the aluminum seal.

Note: With the exception of the diluted ^{125}I Gastrin, all reagents supplied with the kit, and the reagents prepared above, will remain stable for the useful life of the kit with which they were supplied, provided recommended storage conditions are adhered to. Store unopened kits below −15°C.

COLLECTION OF BLOOD SAMPLES FOR ANALYSIS

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 blood sample may be left at room temperature during clotting and removal of the serum. Blood samples that are not processed on the day they are collected, should be stored under refrigeration. Serum samples should be frozen if not assayed within 48 hours of sample collection.

It is recommended that the patient be instructed to fast overnight (i.e., at least 10 hours) prior to collection of the blood sample.

CAUTION: The presence of heparin in the blood sample will alter gastrin binding to the Ion Exchange Resin and thus affect the accuracy of test results.[14]

RADIOIMMUNOASSAY TEST PROCEDURE

Materials Needed

In addition to the reagents and materials supplied with the Gastrin IMMUTOPE Kit, the following equipment is required:

1. Centrifuge capable of 3000-4000 rpm.
2. Well-type gamma scintillation counter
3. Refrigerator
4. Freezer
5. Magnetic stirrer and stirring bars
6. Test tubes, Squibb Standard Test Tube, List #09020
7. Vortex
8. Oxford sampler and tips for 1.0 ml., 100 µl., and 10 µl. (or equivalent)
9. Hamilton repeating syringe and holder, 2.5 ml. to deliver 50 µl., Hamilton Catalog #1002, with 26 g. needles
10. Container for radioactive waste
11. Usual bench equipment including racks, graduates, and beakers of appropriate sizes to accommodate the quantities of reagents used in this test

Important

It will take approximately one hour for the frozen reagents to thaw upon removal from the styrofoam platform. The ^{125}I Gastrin solution is not expected to solidify at the recommended storage temperature.

Repeated thawing and re-freezing of the Gastrin Standards and Gastrin Antiserum do not significantly affect test results.

A smaller clinical sample volume may be used in the radioimmunoassay test procedure if high gastrin levels are anticipated. The calculation of serum gastrin level should be modified accordingly to reflect the aliquot used in the test.

A precipitate may be observed in the Gastrin Standards and Antiserum; however, this does not interfere with test results.

During the separation step, the Ion Exchange Resin Suspension should be added only to the quantity of tubes that can be centrifuged simultaneously. The equilibrium of the antigen-antibody reaction is altered upon prolonged contact with the resin.

To facilitate accuracy in the transfer of the supernatant to its counting tube, it is recommended that the rims of the two tubes be touched together to effect complete transfer of the liquid.

Reagents from one kit should not be intermixed with reagents from other kits.

Procedure

The procedure described below is based on performance of duplicate analyses on all samples assayed. **Read entire procedure before starting test.**

1. Mark a series of test tubes with the numbers 1 through 14. The first 12 tubes are required for preparation of the standard curve and the remaining 2 tubes are required for assay of one clinical sample. Two additional tubes should be used for each additional clinical sample to be assayed.
2. Add 1.0 ml. of diluted ^{125}I Gastrin to each tube.
3. Add the following quantities of Gastrin Standard A to the respective tubes: tubes #3 and #4—10 µl.; tubes #5 and #6—20 µl.; tubes #7 and #8—40 µl.
4. Add the following quantities of Gastrin Standard B to the respective tubes: tubes #9 and #10—10 µl.; tubes #11 and #12—20 µl.
5. Add 100 µl. of the clinical sample to tubes #13 and #14.

The composition of the 14 tubes is summarized below:

Tube #	Sample Added	Gastrin Content (pg.)
1 and 2	None	0
3 and 4	Std A (10 µl.)	5
5 and 6	Std A (20 µl.)	10
7 and 8	Std A (40 µl.)	20
9 and 10	Std B (10 µl.)	50
11 and 12	Std B (20 µl.)	100
13 and 14	Clin. Sample (100 µl.)	unknown

6. Add 50 µl. of Gastrin Antiserum to all 14 tubes, mix gently, and incubate at room temperature (22°-24°C.) for three hours (longer incubation times will not significantly affect test results).
7. At the end of the incubation period add 200 µl. of the Ion Exchange Resin Suspension to all tubes. The suspension should be kept under constant agitation during removal of the 200 µl. aliquots.
8. Mix the sample thoroughly by hand shaking or vortexing for a few seconds, and then centrifuge for 3-4 minutes at 3000-4000 rpm.
9. Decant the supernatant from each tube into correspondingly numbered tubes.
10. Measure the radioactivity in all tubes (i.e., tubes containing resin residue and tubes containing supernatant) by counting in a standard well-type gamma scintillation counter (discriminator settings 20-50 kev). Subtract background cpm and record net cpm for each tube on the data sheet provided with the kit.
11. Calculate the B/F (bound/free) value for each of the original tubes using the following formula and record results on the data sheet:

$$\text{B/F Value} = \frac{\text{Activity in Supernatant (cpm)}}{\text{Activity in Resin (cpm)}}$$

For Example: Tube #1 Supernatant—1975 cpm
Tube #1 Resin —2320 cpm

$$\text{B/F Value (Tube \#1)} = \frac{1975}{2320} = 0.85$$

12. Calculate average B/F values for the duplicate samples and record average values on data sheet.
13. Prepare a *Standard Curve* by plotting the average B/F Values against the quantity of Gastrin Standard added to the respective tubes (i.e., tubes 1 through 12). See the sample standard curve on the data sheet for guidance in plotting the data. The Standard Curve shown on the data sheet is for guidance only, and **should not** be used in calculating gastrin levels in clinical samples.
14. Determine the quantity (pg.) of gastrin in the clinical sample by referring to the Standard Curve prepared

above. The B/F value for each sample will correspond to the specific quantity of gastrin contained in the sample.

15. Calculate the patient serum gastrin level as follows:

Serum Gastrin (pg./ml.) = pg. Gastrin in Sample X 10

For Example:
Clinical sample contains 7.5 pg. gastrin

Serum Gastrin = 7.5 × 10 = 75 pg./ml.

ALTERNATE COUNTING AND DATA PRESENTATION PROCEDURE

Improved counting statistics can be achieved by measuring the total radioactivity (Total Counts) in each tube during the incubation step (Step #6 above), and subsequent to resin addition, centrifugation, and decantation, measurement of the radioactivity in the resin (resin counts) remaining in each tube. While the higher counting rates associated with this approach will improve the accuracy of the test, care must be taken to assure identical instrument settings for the interrupted sequence of radioactivity measurements. If this counting procedure is followed, the B/F value is calculated as follows:

$$\text{B/F Value} = \frac{\text{Total Counts (cpm)} - \text{Resin Counts (cpm)}}{\text{Resin Counts (cpm)}}$$

If desired by the individual laboratory, the Standard Curve can be plotted in terms of % Bound rather than B/F. Using the counting procedure described in the above paragraph, % Bound would be calculated as follows:

$$\%\ \text{Bound} = \frac{\text{Total Counts (cpm)} - \text{Resin Counts (cpm)} \times 100}{\text{Total Counts (cpm)}}$$

RANGE OF EXPECTED VALUES

Normal Values: Using the Gastrin IMMUTOPE Kit, Deodhar and Kumar[15] reported fasting serum gastrin levels of 78.7 ± 31.9 pg./ml., with a range of 50-155 pg./ml. in 30 normal, healthy individuals. In 18 of these individuals, postprandial levels of serum gastrin were found to average 118.3 ± 26.7 pg./ml., with a range of 80-170 pg./ml.

Zollinger-Ellison Syndrome: Using the Gastrin IMMUTOPE Kit, McGuigan[14] reported serum gastrin levels of 260 to greater than 2,000 pg./ml. in nine patients with ZE syndrome, and Deodhar and Kumar[15] reported serum gastrin levels in excess of 2,000 pg./ml. in two patients with ZE syndrome.

Pernicious Anemia: Using the Gastrin IMMUTOPE Kit, Deodhar and Kumar[15] reported a mean serum gastrin level of 912 pg./ml., with a range of 130-2,260 pg./ml. in 15 patients with pernicious anemia.

REPRODUCIBILITY OF TEST RESULTS

Using the Gastrin IMMUTOPE Kit, Deodhar and Kumar[15] reported a mean serum gastrin level of 256 ± 15 pg./ml. for ten replicate assays conducted simultaneously on the same serum specimen, and using a different serum specimen, a mean value of 514 ± 35 pg./ml. for ten assays conducted at different times on aliquots from the same serum sample. Recoveries of added gastrin in the assay procedure were reported to have been in the range of 90-96%.

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