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E. R. Squibb & Sons, Inc.

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New Brunswick, New Jersey 08903
201-545-1300

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U. S. Nuclear Regulatory Commission
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October 27, 1976

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

Nuclear Regulatory Commission
Washington, D.C. 20555

Attention: Mr. Nathan Bassin
Radioisotopes Licensing Branch

Gentlemen:

We hereby wish to supplement our specific license 29-00139-05G, which provides for the distribution of Iodine-125 containing in vitro products to persons generally licensed pursuant to 10 CFR § 31.11, to provide for an additional product, Angiotensin I Immutope Kit; each prepackaged unit does not exceed 10 microcuries of Iodine-125.

Copies of the printed labeling components which contain the information required under § 32.71 (c)(1) and (2), (d), and (e) are enclosed. For the purposes of this supplemental application, only those portions of the brochure, which accompanies the package, containing information pertinent to § 32.71 (d) and (e) are being submitted for review; these are outlined in the attached brochure.

We trust that this information will be adequate and look foward to your approving this supplement.

Sincerely,

C. L. Kroll

C. L. Kroll

/cl
Att.

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INSPECTION AND ENFORCEMENT

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CAUTION
RADIOACTIVE MATERIAL

Medotopes®

List 09402

Angiotensin I IMMUTOPE® Kit

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

Not for Internal or External Use in Humans or Animals

Contains sufficient material for 200 tubes • See enclosed directions

CONTENTS:
- 1 bottle (200 ml) ^{125}I ANGIOTENSIN I BUFFER <1.7 μCi/bottle
- 6 vials (2 ml ea.) ANGIOTENSIN I STANDARD (1 each of 0 pg, 50 pg, 100 pg, 300 pg, and 500 pg/25 μl, respectively)
- 1 vial (10 ml) ANGIOTENSIN I ANTISERUM
- 1 vial (2 ml) ANGIOTENSIN I CONTROL
- 1 vial (210 tabs.) ANGIOTENSIN ADSORBENT CHARCOAL TABLETS
- 1 vial (5 ml) ANGIOTENSIN I PLASMA BUFFER (pH 7.4)
- 1 vial (2 ml) DIMERCAPROL SOLUTION
- 1 vial (330 mg) 8-HYDROXYQUINOLINE SULFATE

STORE KIT BELOW −15° C • ON RECEIPT ANGIOTENSIN I CONTROL AND ANTISERUM CAN BE REMOVED AND STORED IN FREEZER BELOW −15° C • BALANCE OF KIT MAY BE REFRIGERATED BETWEEN 2°–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.

C8076/09402

Shipper Label for Kit
(Expiration Date and Lot No. are Imprinted at the Time of Manufacture)

SQUIBB

CAUTION
RADIOACTIVE MATERIAL

Medotopes®

List 09404

^{125}I ANGIOTENSIN I BUFFER

Approx. 0.005 μg Angiotensin I per 200 ml

For use with Angiotensin I IMMUTOPE® Kit

For *in vitro* Diagnostic Use • See enclosed directions

CONTENTS: 1 Bottle (200 ml) • Sufficient for 200 tubes

Store between 2°–8° C

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

Total Act.: <1.7 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. | C8072/09404

Shipper Label for Buffer
(Shipper is also Labeled with Sticker Label M7678)

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SQUIBB Store between 2°–8° C

CAUTION RADIOACTIVE MATERIAL

200 ml List 09402

^{125}I ANGIOTENSIN I BUFFER

Approx. 0.005 μg Angiotensin I per 200 ml

For *in vitro* Diagnostic Use · See directions

Total Act.: < 1.7 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. C6122/09402

Bottle Label

Not for Internal or External Use in Humans or Animals

M7678

Pressure Sensitive Sticker Label For Each Bottle

SQUIBB

CAUTION RADIOACTIVE MATERIAL

Angiotensin I IMMUTOPE® Kit List 09402

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

Not for Internal or External Use in Humans or Animals

Contains sufficient material for 200 tubes

STORE KIT BELOW −15° C • See enclosed directions

E. R. Squibb & Sons, Inc., Princeton, N.J. 08540

Made in U.S.A. C6113/09402

CONTENTS:

1 bottle (200 ml) ^{125}I ANGIOTENSIN I BUFFER < 1.7 μCi/bottle

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

1 vial (10 ml) ANGIOTENSIN I ANTISERUM

1 vial (2 ml) ANGIOTENSIN I CONTROL

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

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

1 vial (2 ml) DIMERCAPROL SOLUTION

1 vial (330 mg) 8-HYDROXYQUINOLINE SULFATE

Carrier Label for Kit

(Expiration Date and Lot No. are Imprinted at the Time of Manufacture)

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SQUIBB

ANGIOTENSIN I IMMUTOPE R KIT

FOR QUANTITATIVE MEASUREMENT OF PLASMA RENIN ACTIVITY BY RADIOIMMUNOASSAY

For IN VITRO Diagnostic Use
For Professional Use Only

DETERMINATION OF PLASMA RENIN ACTIVITY 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.

Since purified renin is not readily available, the direct radioimmunoassay of renin is not yet practical. In lieu of a direct renin radioimmunoassay, a method has been developed for the estimation of plasma renin activity through the radioimmunoassay of Angiotensin I generated by the action of renin in incubated plasma samples.

In the Angiotensin I IMMUTOPE Kit, antibody to Angiotensin I is raised in the New Zealand white rabbit, against synthetic Angiotensin I coupled to an appropriate carrier. Synthetic Angiotensin I is used as the source for the Angiotensin I Standards, and also serves as the starting material for the ^{125}I Angiotensin I. Charcoal is used to separate free Angiotensin I from antibody-bound Angiotensin I. Plasma renin activity (PRA) measured in this manner is expressed in terms of nanograms (ng., 10^{-9}g.) of Angiotensin I generated per milliliter of plasma per hour of incubation at 37°C (ng./ml./hr.).

RATIONALE FOR USE

The radioimmunoassay for Angiotensin I and its application to the determination of plasma renin activity has been of great value in studying physiological processes associated with hypertension,[1-8] and has become increasingly important in the clinical evaluation of hypertensive disease.[9-15] The measurement of plasma renin activity by radioimmunoassay has several advantages over the standard bioassay procedures. The simplicity, specificity, and rapidity with which the radioimmunoassay can be performed remove the obstacles to routine clinical determination of plasma renin activity that were associated with the more complex bioassay procedure.

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The use of ^{125}I rather than ^{131}I considerably lengthens the shelf-life of the radioiodinated Angiotensin I 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.

RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

Renin is an enzyme that is synthesized, stored, and released from granules contained in the juxtaglomerular apparatus of the kidney. It is generally accepted that the major physiological role of renin is to act as the primary stimulus for maintenance of body sodium balance. Plasma renin activity is increased when there is a decreased renal perfusion pressure, and when there is a decreased delivery of sodium and water to the distal tubule. Renin acts on a substrate, angiotensinogen, an α-2-globulin produced by the liver, to form a decapeptide, Angiotensin I. Angiotensin I is biologically inactive, and is converted to the biologically active octapeptide, Angiotensin II, in the pulmonary circulation. Angiotensin II acts as a highly potent vasopressor, and stimulates the adrenal gland to produce aldosterone. Aldosterone promotes the reabsorption of sodium by the distal tubule, and when secreted in excessive amounts, results in hypertension.

CLINICAL APPLICATIONS

The measurement of plasma renin activity (PRA) may be a useful adjunct in determining whether hypertension is due to primary aldosteronism or to renal vascular disease. The hypertensive patient with a low peripheral PRA, and high aldosterone secretion rate, in whom there is a consistent suppressed PRA in response to maintenance of upright posture, restricted salt intake, and oral administration of a diuretic drug, will usually be found to have adrenal hyperplasia or an adenoma of the zona glomerulosa of the adrenal gland. In hypertensive patients with renal artery stenosis, the renal venous blood from the ischemic kidney may be found to have higher PRA than renal venous blood from the unaffected kidney.

CLINICAL BACKGROUND

Using a radioimmunoassay procedure, Page [16] reported resting PRA values of 0.56 ± 0.41 ng./ml./hr. in 44 essential hypertensive patients. Blood samples were withdrawn with the patient in the supine position after an overnight rest with no dietary salt restriction, and after being off medication for at least five days. No adjustment of plasma pH was made during the 37°C plasma generation step.

It is important to note that Cohen et al,[17] using their technique, have reported normal values of 2.5 ± 1.4 ng./ml./hr. for normal volunteers in the supine position. The significant difference in the normal values obtained by Cohen et al, from that obtained by Page, is related primarily to the pH of the incubation medium during the generation of Angiotensin I. The diagnostic significance of the greater generation of Angiotensin I using a pH 5.5 incubation, versus the non-pH adjusted plasma incubation, is yet to be established.

Brunner et al,[18] reported a reproducibility of ± 14 percent (S.D.) for the bioassay procedure previously used in their laboratory, and a reproducibility of ± 12 percent (S.D.) for the currently used radioimmunoassay procedure. By applying a correction factor to their bioassay results that accounts for the fact that Angiotensin I is not as pressor by weight as Angiotensin II, the bioassay and radioimmunoassay results were completely interchangeable over the entire physiologic range.

Important: Many factors are known to influence plasma renin activity. Such factors include the posture of the patient, the sympathetic nervous system, and certain drugs, particularly diuretic agents. Plasma renin activity is increased by sodium depletion, upright posture, pregnancy, anovulatory drugs, secondary aldosteronism, in the malignant phase of hypertension, and in some cases of renal hypertension.[12,19-22] In addition to the above, it has been found that all the hypertensive agents studied thus far significantly influence plasma renin activity. Antihypertensive drugs, including diazoxide, reserpine, and hydralazine have been shown to increase plasma renin activity.[23,24]

Plasma renin activity is decreased in primary aldosteronism and during drug therapy with methyldopa, L-dopa, and guanethidine.[25,26]

From the above discussion, it is quite evident that a noncontrolled determination of plasma renin activity on an ambulatory patient may not be clinically useful. The significance of plasma renin activity measurements can be meaningfully interpreted only when the patient is studied under controlled conditions and with defined sodium balance.

REAGENTS

The Angiotensin I IMMUTOPE Kit is available in a 200-test package. Each kit contains one bottle of ^{125}I Angiotensin I Buffer (200 ml., with a total activity of <1.7 μCi) six vials of Angiotensin I Standard (2 ml. each; 0 pg, 50 pg, 100 pg, 200 pg, 300 pg, 500 pg), one vial of Angiotensin I Antiserum (10 ml.), one vial of Angiotensin I Control (2 ml.), one vial of Angiotensin I Adsorbent Charcoal Tablets (210 tablets), one vial of Angiotensin I Plasma Buffer (5 ml., pH 7.4), one vial of Dimercaprol Solution (2 ml.), and one vial of 8-Hydroxyquinoline Sulfate (330 mg.).

In addition to the complete kit described above, ^{125}I Angiotensin I Buffer, Angiotensin I Standard, Angiotensin I Antiserum and Angiotensin I Control can be purchased individually.

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. Nuclear Regulatory Commission or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority.

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E.R. Squibb & Sons, Inc.

In vitro clinical laboratory testing with the Angiotensin I IMMUTOPE kit requires only a general license from the U.S. Nuclear Regulatory Commission. The general license is issued to any physician, clinical laboratory, or hospital who obtains a validated registered U.S.N.R.C. Form 483. This form must be submitted in triplicate to the U.S.N.R.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 U.S.N.R.C. for quantities larger than 200 μCi).

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 the decay can no longer be measured.

IMPORTANT NOTE: The commonly used bacteriostatic agent, sodium azide, that is used in most of the reagents supplied with the Angiotensin I IMMUTOPE Kit, has been implicated in laboratory explosions when this material was disposed of through the laboratory plumbing system. To avoid the formation of highly explosive copper and lead azides, it is recommended that excess reagents and assay samples that are disposed of through the laboratory plumbing system be thoroughly flushed with large amounts of water.

PREPARATION AND STORAGE OF TEST REAGENTS

8-Hydroxyquinoline Sulfate Solution: Add 5 ml. of distilled water to the vial containing 8-Hydroxyquinoline sulfate (380 mg.) and shake gently to dissolve the solids. Store in the Dark at 2 - 8°C.

The Angiotensin I Antiserum and Angiotensin I Control must be stored at -15°C. The other components of the kit may be stored at either 2 - 8°C or -15°C, whichever is more convenient.

COLLECTION OF BLOOD SAMPLES FOR ANALYSIS

Blood should be collected in a cold tube containing EDTA. B-D Vacutainer tube, lavender stopper, Catalog # 4770, 4739, 4713 (disodium edetate), or 4784, 4759, 4727 (tripotassium edetate) may be used. The blood samples should be stored under refrigeration, and centrifuged in the cold to collect the plasma. If the assay is not conducted within 24 hours of collection, all plasma samples should be frozen until used.

RADIOIMMUNOASSAY TEST PROCEDURE

Materials Needed

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

1. Centrifuge capable of 3000-4000 rpm
2. Well-type gamma scintillation counter
3. Refrigerator
4. Freezer
5. Vortex mixer
6. Test tubes, 12 x 75 mm polystyrene
7. Pipettor and tips for 1.0 ml., 0.5 ml., and 25 µl.
8. Hamilton repeating syringes and holders, 0.5 ml to deliver 10 µl., and 2.5 ml. to deliver 50 µl. (or equivalent)
9. Container for radioactive waste
10. Usual bench equipment including racks, graduates, and beakers of appropriate sizes to accommodate the quantities of reagents used in this test.

Important

Frozen reagents should be completely thawed prior to use. The use of a water bath will accelerate the thawing process. With the exception of the Angiotensin I Control, repeated thawing and refreezing of the Angiotensin I IMMUTOPE Kit reagents will not significantly affect test results. To facilitate use of the Angiotensin I Control, it is recommended that the material be thawed and appropriate aliquots be transferred to suitable test tubes and frozen for subsequent use.

With the exception of the Angiotensin I Control, all of the reagents supplied in the Angiotensin I IMMUTOPE Kit should be stored in their original containers, with their original closure. Do not transfer these reagents to other containers or change the closures supplied with the vials.

Strict attention should be given to the time and temperature of the plasma incubation (generation of AI in clinical samples) step, as the quantity of AI generated is somewhat sensitive to these parameters.

Assay results will be non-linear if there is deviation from the recommended 25 µl. sample size. If AI levels must be measured that exceed the sensitive portion of the standard curve, the incubated sample should be diluted with an unincubated aliquot of the patient's plasma. The contribution of the circulating AI level in the unincubated plasma should be subtracted from the final assay result. If extremely low levels must be measured, assay reliability can be improved by extending the time of the 37°C incubation. Linear AI generation has been observed for periods from 2 to 6 hours. The calculation of PRA should be modified accordingly to reflect the generation time used.

During the separation step, the charcoal tablets 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 tablet, samples should be centrifuged as quickly as possible, and not allowed to stand at room temperature. It is not essential that a refrigerated centrifuge be used.

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 for Incubation of Plasma Samples to Generate Angiotensin I

1. To 1.0 ml. of plasma, collected in the manner described above, add 10 microliters (µl.) of 8-Hydroxyquinoline Sulfate Solution, and 10 µl. of Dimercaprol Solution, and mix gently, but thoroughly.

2. Transfer 0.5 ml. of the above mixture to another test tube and add 50 µl. of Angiotensin I Plasma Buffer to this tube only. Mix well.

3. Incubate the unbuffered plasma (residual plasma remaining in Step #1) at 4°C for 2 hours. This sample will reflect circulating levels of Angiotensin I.

4. Incubate the buffered plasma (prepared in Step #2) at 37°C for 2 hours. This sample will reflect circulating levels plus the quantity of Angiotensin I generated through the action of renin in the plasma sample. The net quantity of Angiotensin I generated at 37°C is calculated by subtracting the Angiotensin I level in the 4°C sample from the Angiotensin I level in the 37°C sample.

   Note: If the incubated samples are not used within 24 hours, in the radioimmunoassay-procedure described below, the samples should be frozen until used.

Radioimmunoassay Procedure

The procedure described below is based on the 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 18. The first 12 tubes are required for preparation of the standard curve, the next 2 tubes are required for the Angiotensin I Control sample, and the remaining 4 tubes are required for assay of one clinical sample. Four additional tubes should be used for each additional clinical sample to be assayed.

2. Add 1.0 ml. of ^{125}I Angiotensin I Buffer to each tube.

3. Add the following quantities of Angiotensin I Standard to the respective tubes: Tubes 1 and 2 - 25 µl. of 0 pg. Standard; Tubes 3 and 4 - 25 µl. of 50 pg. Standard; Tubes 5 and 6 - 25 µl. of 100 pg. Standard; Tubes 7 and 8 - 25 µl. of 200 pg. Standard; Tubes 9 and 10 - 25 µl. of 300 pg. Standard; Tubes 11 and 12 - 25 µl. of 500 pg. Standard.

4. Add 25 µl. of Angiotensin I Control to Tubes 13 and 14.

5. Add 25 µl. of the clinical sample incubated at 4°C to Tubes 15 and 16, and 25 µl. of the clinical sample incubated at 37°C to Tubes 17 and 18.

The composition of the 18 tubes is summarized below:

Tube #	Sample Added	AI Content (pg.)
1 and 2	25 µl. 0 pg. Std.	0
3 and 4	25 µl. 50 pg. Std.	50
5 and 6	25 µl. 100 pg. Std.	100
7 and 8	25 µl. 200 pg. Std.	200
9 and 10	25 µl. 300 pg. Std.	300
11 and 12	25 µl. 500 pg. Std.	500
13 and 14	25 µl. AI Control	See Label Assay
15 and 16	25 µl. 4°C Clin. Sample	Unknown
17 and 18	25 µl. 37°C Clin. Sample	Unknown

6. Add 50 µl. of Angiotensin I Antiserum to all tubes, mix gently, and incubate in refrigerator (4°C) for 24 ± 2 hours.

7. During the incubation period, measure the radioactivity in two tubes to obtain a value for total radioactivity (TOTAL COUNTS) for subsequent use in the calculation of % Bound. Note: <u>This measurement must be made prior to the addition of the Angiotensin I Adsorbent Charcoal Tablets</u>.

8. At the end of the incubation period add one Angiotensin I Adsorbent Charcoal Tablet to all tubes and vortex for at least 5 seconds. Centrifuge the tubes for 5 - 7 minutes at 3000 rpm.

9. Decant the supernatant from each tube into similarly numbered tubes. Discard the charcoal residue to radioactive waste.

10. Measure the radioactivity in all the supernatant tubes (SUPERNATANT COUNTS) 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 each kit.

RESULTS

% Bound vs. pg. AI (Linear-Linear) Plot

1. Calculate the % Bound for each of the tubes using the following formula, and record the results on the worksheet:

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

Example:

Background cpm: 250
Tube #1 SUPERNATANT COUNTS (gross cpm): 6550 cpm
TOTAL COUNTS (gross cpm): 11,750 cpm

$$\% \text{ Bound} = \frac{6550 - 250}{11{,}750 - 250} \times 100$$

$$= \frac{6300}{11{,}500} \times 100 = 54.8\%$$

2. Calculate average % Bound for the duplicate samples and record the average values on the worksheet.

3. Prepare a Standard Curve by plotting the average % Bound values against the quantity of Angiotensin I 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 Angiotensin I levels in clinical samples.

4. For individual laboratory quality control purposes, determine the quantity of Angiotensin I in the Angiotensin I Control Sample (tubes 13 and 14) by referring to the standard curve prepared above. The average % Bound will correspond to the specific quantity of Angiotensin I contained in the sample. The quantity measured should be in reasonably good agreement with the quantity specified on the Angiotensin I Control label.

5. Determine the quantity (pg.) of Angiotensin I in the 4°C sample and the 37°C sample by referring to the standard curve prepared above. The % Bound for each sample will correspond to the specific quantity of Angiotensin I contained in the sample.

6. Calculate plasma renin activity (PRA) in terms of nanograms of Angiotensin I generated per ml. of plasma per hour of incubation at 37°C as follows:

   PRA (ng./ml./hr.) = (pg. AI in 37°C Sample - pg AI in 4°C Sample) X 0.022*

   Example: 37°C sample contains 160 pg. AI
   4°C sample contains 10 pg. AI

   PRA = (160 - 10) X 0.022 = 3.3 ng./ml./hr.

* Factor to correct for sample dilution, plasma incubation time, and units conversion.

B/B$_0$ vs. pg. AI (Semi-Log) Plot

1. Calculate B/B$_0$ for each of the tubes using the following formula:

$$B/B_0 = \frac{\text{SUPERNATANT COUNTS (net cpm)}}{\text{Avg. SUPERNANT COUNTS of "0" pg. Std (net cpm)}}$$

Example: Background cpm: 250
Supernatant Counts (patient sample)(net cpm): 3750
Avg. Supernant Counts ("0" pg. Std.)(net cpm): 6550

$$B/B_0 = \frac{3750 - 250}{6550 - 250}$$

$$= \frac{3500}{} = 0.55$$

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2. Calculate average B/B_0 for the duplicate samples.

3. Prepare a Standard Curve by plotting average B/B_0 values against the log of the quantity of Angiotensin I 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 Angiotensin I levels in clinical samples.

4. For individual laboratory quality control purposes, determine the quantity of Angiotensin I in the Angiotensin I control sample (tubes 13 and 14) by referring to the standard curve prepared above. The average B/B_0 will correspond to the specific quantity of Angiotensin I contained in the sample. The quantity measured should be in reasonably good agreement with the quantity specified on the Angiotensin I Control label.

5. Determine the quantity (pg.) of Angiotensin I in the 4°C sample and the 37°C sample by referring to the standard curve prepared above. The B/B_0 for each sample will correspond to the specific quantity of Angiotensin I contained in the sample.

6. Calculate plasma renin activity (PRA) by the formula given in Step 6 under Results, % Bound vs. pg. AI (Linear-Linear) Plot.

EXPECTED VALUES

It is critically important to recognize that a great many factors are known to influence plasma renin activity, and thus normal ranges should be established in each laboratory for the specific testing protocol followed. Using the Angiotensin I IMMUTOPE Kit, the following results were obtained by four investigators studying normal volunteers:

	Plasma Renin Activity (ng./ml./hr.)	
Normal Volunteers	upright	supine
17 volunteers	1.90 ± 0.65	0.98 ± 0.36
21 volunteers	3.08 ± 1.27	1.58 ± 0.92
20 volunteers	2.53 ± 1.21	1.24 ± 0.65
28 volunteers	1.75 ± 1.12	-

Plasma renin activity measurements were performed by two investigators on 5 anephric patients. In the supine position, PRA meaurements ranged from 0.15 - 0.70 ng./ml./hr., and in the upright position ranged from 0.3 - 0.49 ng./ml./hr. PRA meaurements in the same patients using a pH 5.5 plasma incubation procudure, yielded PRA values that were almost twice that observed with the pH 7.4 plasma incubation employed in the Angiotensin I IMMUTOPE Kit.

PARAMETERS OF THE TEST PROCEDURE

The pH of the incubation medium during the generation of Angiotensin I has been shown to be of considerable significance on the amount of Angiotensin I generated at 37°C. It has been generally accepted that maximum generation of Angiotensin I is achieved at pH 5.5 - 6.0, but some concern has been expressed as to whether Angiotensin I generation in this pH range is totally due to the action of renin. Contrary to literature reports on the pH dependence of Angiotensin I generation, the Angiotensin I IMMUTOPE Kit employs a pH 7.4 buffer that yields an Angiotensin I generation rate that is essentially equivalent to that observed with pH 5.5 - 6.0 incubation. The exact mechanism by which the Angiotensin I Plasma Buffer exerts this enhanced effect on Angiotensin I generation has not been established. The higher level of Angiotensin I generation is of special value in measuring plasma renin activity in patients with low renin levels.

The specificity and sensitivity of the test procedure depends on the specific characteristics of the antiserum, and to some extent the tagged antigen, provided in the individual kit shipments. The antiserum provided in the Angiotensin I IMMUTOPE Kit shows no measurable cross-reactivity with Angiotensin II. The sensitivity of the assay system, based on the lowest detectable quantity of Angiotensin I, is 10 pg. of Angiotensin I in the 25 µl. sample size, which corresponds to a PRA of 0.2 ng./ml./hr. The affinity constant for the antiserum is calculated to be 1.4×10^{10} liters/Mole.

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, Angiotensin I IMMUTOPE Kit results have shown an intraassay coefficient of variation (CV) of 5 - 8% for samples with a PRA of less then 7 ng./ml./hr. and 15% for samples with a higher PRA.

An interassay CV of 10% was observed for samples with a PRA below 7 ng./ml./hr., and 20% for higher PRA samples.

Studies on the recovery from plasma of added Angiotensin I have been conducted with the Angiotensin I IMMUTOPE Kit over a wide range of Angiotensin I concentrations and with plasma samples from a variety of patients. Recoveries of 90 - 100% were observed.

A very high degree of correlation has been reported between results obtained by bioassay and radioimmunoassay procedures. On a series of 91 determinations, using the Angiotensin I IMMUTOPE Kit and a standard bioassay method, a regression coefficient of 0.9096 was obtained.[27]

It will take a total time of approximately 26 hours to perform the assay procedure. The incubation time and temperature for the plasma generation step are critical, but time and temperature of the antigen-antibody incubation can be varied between 22 - 26 hours and 2 - 8°C, respectively. Minor variations in the individual steps of the separation procedure (e.g., charcoal contact time before, during, and after vortexing), have been shown to have little effect on assay results.

The Angiotensin converting enzyme inhibitors(i.e., 8-Hydroxyquinoline and Dimercaprol) used in the Angiotensin I IMMUTOPE Kit in addition to EDTA, have been shown to be as effective as other commonly used inhibitors (e.g., PMSF, DFP) for the plasma incubation conditions employed in the test procedure.

REFERENCES

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