I. INTRODUCTION

A. PHYSIOLOGY OF THE RENIN-ALDOSTERONE AXIS

Aldosteronism, produced by the zona glomerulosa of the adrenal gland, is the major sodium retaining hormone in the body. Minuten increases in sodium-potassium exchange at the distal tubule of the kidney, resulting in sodium retention and urinary loss of potassium. The serum level of potassium (K) is controlled by a number of factors, most importantly being the serum potassium level and the systems for excretion of potassium. Sodium and potassium are excreted by the kidney. 

Renin is a peptide hormone produced by the juxtaglomerular cells of the kidney. A number of physiological stimuli lead to increased renin release. These include salt deficiency, stimulation of the sympathetic nervous system, the renin-angiotensin-aldosterone system, activation of angiotensin converting enzyme (ACE), and A/Ps in normal individuals will have a decrease in sodium-potassium exchange at the distal tubule of the kidney, resulting in sodium retention and urinary loss of potassium. The serum level of potassium (K) is controlled by a number of factors, most importantly being the serum potassium level and the systems for excretion of potassium. Sodium and potassium are excreted by the kidney.

II. PRINCIPLE OF THE TEST

Radioimmunoassay (RIA) is the term applied to the measurement of the concentration of antigen molecules using a radioisotope label that quantifies the amount of antigen (i.e., hormone) by determination of the extent to which it combines with its antibody.

The assay, a limited amount of specific antibody (Ab) is reacted with the concentration of antigen (A) in the sample. The antigen molecules may not be an amount of hormone (H), a corresponding decreasing fraction of “A” is added to the bound to the antibody. The antigen molecules may not be recognized by the antibody, then the amount of radioactivity in one or both of these two fractions is evaluated and used to construct a standard curve.

III. REAGENTS PROVIDED AND LABEL COLOR CODE (100 Tube Kit)

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>LABEL COLOR</th>
<th>VOLUME OR QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat # 07-166624</td>
<td>Yellow</td>
<td>11 mL</td>
</tr>
<tr>
<td>Cat # 07-108230</td>
<td>Blue</td>
<td>11 mL</td>
</tr>
<tr>
<td>Cat # 07-118224</td>
<td>Tan</td>
<td>2 x 5 mL</td>
</tr>
<tr>
<td>Cat # 13-061624</td>
<td>Red</td>
<td>50 mL</td>
</tr>
</tbody>
</table>

IV. REAGENTS DESCRIPTION AND PREPARATION FOR RIA (HORMONE DIAGNOSTIC USE)

A. ANTI-ALDOSTERONE

Aldosterone-3-3-lodo-methyliodide: SIA was used as the antigen to generate antibodies in rabbits. The antiserum (1/50 dilution) against the -ion of the antiserum. The antiserum has been diluted with steroid and used as the antibody in the assay.

STORAGE: 2 to 8°C

B. ALDOSTERONE STANDARDS

Seven standards are provided at the following concentrations: 1.0, 2.5, 5.0, 10, 25, 50, 100 ng/mL. The standards have been diluted with steroid and used as the antibody in the assay.

STORAGE: 2 to 8°C

C. ALDOSTERONE*2

This standard contains less than 3 ng/mg per ml to a 100 ng/mg of this radiolabeled material will provide approximately 30,000 cpm at 75% counter efficiency in the assay medium of this tracer. The material contains sodium and potassium.

STORAGE: 2 to 8°C

D. STEROID DILUENT

Phosphate buffered saline, pH 7.8 containing rabbit gamma globulins and 5% glycine.

STORAGE: 2 to 8°C

E. PRECIPITATION SOLUTION

A combination of goat-antibody-gamma globulins and PEG are contained in the assay. 0.5 mL of the solution will immediately precipitate all the antibody bound antigen.

STORAGE: 2 to 8°C

F. BIBLIOGRAPHY

1. This radioactive material may be received, acquired, possessed and used only by those who are licensed or certificated by the appropriate state to do so, by the Internal and External Affairs. The materials, i.e. radio-activity is not transferred, derivative use and transfer are subject to the regulations of the state, and with a general license from the state, with the state with whom the U.S. NRC has entered into agreement for the exercise of regulatory authority.

2. Immediately upon receipt of the kit, check for breakage and verify the contents of the assembled kit. Should any of the breakage or questions regarding the quality of the kit arise, the kit should be returned to the laboratory for examination.

3. Kits required should be stored and used only at designated work stations. The laboratory. Any exposure to the sample from the external atmosphere. It is in a good position to designate a storage area at least 10 feet from any work station. Furthermore, personnel under the age of 18 should not be permitted to handle radiochemicals. Any personnel who are not adequately trained may be hazardous.

4. Should contamination of the radioactive material, the following clean-up procedure is recommended: while wearing gloves, wipe the spill with the least radioactivity as possible, then wash your hands with soap and water.

5. The pipetting of radioactive material by mouth should be avoided. Smoking, eating or drinking while performing tests involving radioactive material should not be permitted. Lately, people handling radioactive material should wash their hands with soap and water immediately before eating or drinking.

6. Withdraw 0.5 mL aliquots for assay. (Note: each 0.5 mL is equivalent to 1 µL of the kit).

V. LIMITATIONS, PRECAUTIONS AND GENERAL COMMENTS

1. There are no limitations or precautions to this kit.

2. There are no general comments to this kit.

VI. SPECIMEN COLLECTION AND HANDLING

Plasma: Draw blood into a green capped (heparin) vial. Separate the plasma by centrifugation and store in a refrigerator (up to 1 week) or at room temperature.

Sera: Draw blood into a red capped vial. Allow the blood to clot at least 1 hour. Centrifuge the specimen and store in serum or store under the same conditions as the plasma.

Notes: Aldosterone in light tight, microwave-resistant exposure of serum/plasma samples (or sample extracts) to any light source.

VII. EQUIPMENT AND REAGENTS REQUIRED

6. After the above incubation, place tubes in a 4°C water bath and incubate for SIXTY (60) MINUTES.

7. Vortex all tubes thoroughly and incubate at room temperature for SIXTY (60) MINUTES.

8. Withdraw 0.5 mL aliquots for assay. (Note: each 0.5 mL is equivalent to 1 µL of the kit).

9. Proceed to WBC, to assess the quality of the kit.

Q. QUALITY CONTROL

A. Average the counts of duplicate tubes. Subtract the averaged blank (NSB) counts by the corrected zero standard counts to obtain the percent bound at all tubes. Calculated results should be less than the sensitivity of the standard response.
### B. Sodium Diet

- Control HB
- B12/C12

#### XII. SAMPLE STANDARD CURVE

<table>
<thead>
<tr>
<th>Sample</th>
<th>CPM 10</th>
<th>CPM 30</th>
<th>CPM 40</th>
<th>CPM 50</th>
<th>CPM 80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>7507</td>
<td>7466</td>
<td>6793</td>
<td>6963</td>
<td>14.5 X 10^6 = 62</td>
</tr>
<tr>
<td>Control 2</td>
<td>3342</td>
<td>3421</td>
<td>2748</td>
<td>2842</td>
<td>1 X 10^6 = 145</td>
</tr>
<tr>
<td>Control 3</td>
<td>5288</td>
<td>5464</td>
<td>4791</td>
<td>4914</td>
<td>5.1 X 10^4 = 420</td>
</tr>
</tbody>
</table>

100 pg/tube: 2485, 2443, 1770, 1850
50 pg/tube: 3122, 3125, 2452, 2530
25 pg/tube: 4417, 4419, 3746, 3824
10 pg/tube: 6254, 6256, 5577, 5657
2.5 pg/tube: 8915, 9017, 8344, 8504
1 pg/tube: 9676, 9863, 9190, 9350
0 pg/tube: 10531, 10509, 9836, 10000

**NOTE:** Values for samples that fall either higher or lower than the standard curve should not be determined by extrapolation.

#### CALCULATIONS FOR URINARY ALDOSTERONE

pg (read from std curve) x 10 = pg/mL of urine

### A. Parallelism

- The following ligands have been checked for cross reactivity with this antiserum. The percentages indicate the cross-reactivity at 50% displacement compared to the aldosterone curve.

#### B. Recovery

To determine the accuracy of the method, known amounts of aldosterone were added to aliquots of three serum samples, previously assessed.

#### C. Patient Sample Correlation

### D. Intra-Assay Variation

#### E. Inter-Assay Variation

### XIV. Performance Characteristics

- **A. Parallelism**
- **B. Recovery**
- **C. Patient Sample Correlation**
- **D. Intra-Assay Variation**
- **E. Inter-Assay Variation**

### XV. Specificity of the Antiserum

- The following ligands have been checked for cross reactivity with this antiserum. The percentages indicate the cross-reactivity at 50% displacement compared to the aldosterone curve.

#### XV. References