Analytical Performance

Plasma renin activity (PRA), aldosterone, cortisol and adrenocorticotropic hormone (ACTH) were measured using the commercial immunoassay kits.

**Plasma Renin Activity**
Plasma renin activity is quantitated by measuring the rate of angiotensin 1 generation during incubation of plasma renin with endogenous renin substrate. The angiotensin is quantitated by radioimmunoassay.

Assay sensitivity is an important issue, since approximately 30% of patients with essential hypertension have subnormal plasma renin activity. The assay system for angiotensin 1 is sensitive with a detection limit of 0.1 ng/mL/hr (reference interval : 0.2~2.7 ng/mL/hr). Also, the assay system is very specific to angiotensin 1.

Cross reactivities of related peptides in the assay are as follows:
- angiotensin 2 or angiotensin 3<0.0024%, Asn¹ Val⁵/Sar¹ Thr⁸/Sar¹ Ala⁸/Sar¹ Ile⁸-angiotensin 2<0.0012%.

Intra-assay precision of the assay was determined by assaying (n=20) quality-control pools with low, middle, and high concentrations on the same day. The following data were obtained : 7.0~9.2%.

Inter-assay precision of the assay was determined by assaying the same control pools over a period of 10 days. The following data were obtained : 7.5~13.6%.

**Aldosterone**
Aldosterone is measured directly by radioimmunoassay, without extracting the target analyte.

A detection limit of the assay is 7 pg/mL.

The antiserum used in this assay cross reacted little with other structurally related steroids:
- corticosterone=0.03%, 18-OH corticosterone=0.01%, cortisol=0.0002%, cortisone<0.0003%,
- deoxycorticosterone=0.05%, dexamethasone<0.00003%, prednisolone<0.00003%,
- spironolactone=0.0004%, 18-OH deoxycorticosterone=0.04%, progesterone=0.008%,
- 18-OH progesterone=0.005%, and tetrahydrocorticosterone<0.0003%.

Intra-assay and inter-assay precisions of the assay was determined in the same way as a case of PRA. Intra-assay precision: 5.0~7.8%, inter-assay precision: 5.7~10.6%.

**Cortisol**
Cortisol is measured by fully automated immunoassay system, Beckman-Coulter's UniCel Dxi 800.

A detection limit of the assay is 0.4 μg/dL.

The following data indicates the cross reactivity of the assay with steroid substances that are similar in structure to cortisol:
- corticosterone=2.08%, cortisone=8.06%, deoxycorticosterone=0.91%, 11-deoxycortisol=17.80%,
- 17alpha-hydroxyprogesterone=5.33%, progesterone=0.46%, tetrahydrocortisone=0.10%,
- prednisolone=7.60%, prednisone=3.05%, dexamethasone=0.04%

Intra-assay and inter-assay precisions of the assay was determined in the same way as above.
Intra-assay precision: 5.4～6.3%, inter-assay precision: 3.9～6.0%.

**ACTH**

Adrenocorticotropic hormone (ACTH) is measured by electrochemiluminescence Elecsys immunoassay on the Modular Analytics E170 immunoassay analyzer. The assay detection limit is 1.0 pg/mL.

The Elecsys ACTH two-site immunoassay measures intact ACTH 1-39. When ACTH fragments or peptides were added to a patient’s plasma sample with defined ACTH concentration, no interference was observed with ACTH 1-10, ACTH 11-24, beta-MSH, and beta-endorphin. Other ACTH fragments (ACTH 1-17, ACTH 1-24, ACTH CLIP 18-39, ACTH 22-39, alpha-MSH 1-13) >approximately 5,000 pg/mL can bind to one of the antibodies and thereby negatively interfere with the sandwich formation and lead to lower ACTH values. Besides, pro-opiomelanocortin (POMC) showed approximately 1.6% cross-reactivity at 1,560 pmol/L.

Intra-assay and inter-assay precisions of the assay was determined in the same way as above. Intra-assay precision: 1.9～4.0%, inter-assay precision: 3.9～4.2%.

All assays were handled according to manufacturer’s instructions in all respects by experienced technicians in a routine laboratory setting. Quality control was performed for all analytical runs using human plasma pools (PRA and aldosterone) or commercially available quality-control materials (cortisol and ACTH).

Analyte stability was evaluated by healthy volunteer samples after storage up to 12 weeks at refrigerated (4°C) and frozen (-20°C) conditions, respectively. PRA is stable up to 4 weeks at frozen, aldosterone and ACTH are up to 12 weeks at frozen, and cortisol is up to 12 weeks at refrigerated.