

Department:	Laboratory and Blood Bank (Hormone)		
Document:	Internal Policy and Procedure		
Title:	Analysis of Adrenocorticotrophic hormone Level		
Applies To:	All Laboratory Staff		
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1. PURPOSE:

- 1.1 To illustrate the necessary steps required for performing ACTH assay on COBAS e411.

2. DEFINITONS:

- 2.1 Adrenocorticotrophic hormone (ACTH) or corticotropin is a peptide hormone consisting of 39 amino acids. It is produced in the anterior pituitary of the brain as part of the precursor molecule pro-opiomelanocortin (POMC). Tissue-specific cleavage results in ACTH and a range of related peptides. ACTH stimulates formation and secretion of glucocorticoids (especially cortisol) by the adrenal cortex.

3. POLICY:

- 3.1 The glucocorticoid production is regulated by various factors. After stimulation (e.g. by physical effort or by the internal body clock), the hypothalamus secretes CRH (corticotropin releasing hormone). CRH acts on the pituitary, which in turn synthesizes and secretes ACTH. Finally, ACTH stimulates secretion of the glucocorticoids by the adrenals. High concentrations of glucocorticoids in the blood inhibit secretion of CRH and ACTH via a negative feedback mechanism.
- 3.2 ACTH concentrations show a diurnal variation with high levels in the morning and low levels in the evening. Therefore, as with cortisol, it is important to know the collection time of the plasma sample for interpretation of the results.

4. PROCEDURE:

- 4.1 **Principle:** Sandwich principle (for details refer to Company Leaflets of reagents).
- 4.2 **Sample:** K2- and K3-EDTA plasma, collected using siliconized glass tubes or plastic tubes as ACTH adsorbs to non-siliconized glass tubes and thereby reduces sample ACTH values. Do not use other types of plasma samples. Criterion for K2-EDTA plasma: slope 0.85-1.15 for method comparison vs K3-EDTA plasma. Only use pre-cooled sampling vials. After drawing the blood, put the vials immediately on ice. Use a cooled centrifuge to separate the plasma. Measure samples immediately or freeze them at -20 °C (± 5 °C). Stable for 2 hours at 22 °C, 4 weeks at -20 °C (± 5 °C). Freeze only once.
- 4.3 **Method:** See policy of loading sample on machine (Ref: Operative Manuals' of COBAS e411).
- 4.4 **Calculation:** The analyser automatically calculates the analytic concentration of each sample in pg/mL.
- 4.5 **Status:** Stat and Routine.
- 4.6 **Reference ranges:** 7.2-63.3 pg/mL (1.6-13.9 pmol/L) The plasma samples were drawn between 7-10 a.m. ACTH concentrations vary considerably depending on physiological conditions. Therefore, ACTH results should always be evaluated together with simultaneously measured cortisol concentrations.
- 4.7 **Limitations- interference:**
 - 4.7.1 The assay is unaffected by icterus (bilirubin < 428 µmol/L or < 25 mg/dL), hemolysis (Hb < 0.25 mmol/L or < 0.4 g/dL), lipemia (Intralipid < 1500 mg/dL) and biotin (< 246 nmol/L or < 60 ng/mL).

- 4.7.2 Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.
- 4.8 Measuring range: 1.00-2000 pg/mL or 0.220-440 pmol/L (defined by the lower detection limit and the maximum of the master curve).
 - 4.8.1 Values below the lower detection limit are reported as < 1.00 pg/mL or < 0.220 pmol/L.
 - 4.8.2 Values above the measuring range are reported as > 2000 pg/mL or > 440 pmol/L.

5. MATERIALS AND EQUIPMENT:

5.1 Reagent: For preparation see package insert.

- 5.1.1 M: Streptavidin-coated micro particles (transparent cap), 1 bottle, 12 mL: Streptavidin-coated microparticles 0.72 mg/mL, preservative.
- 5.1.2 R1: Anti-ACTH-Ab~biotin (gray cap), 1 bottle, 8 mL: Biotinylated monoclonal anti-ACTH antibody (mouse) 0.3 mg/L; MESb₂ buffer 50 mmol/L, pH 6.2; preservative
- 5.1.3 R2: Anti-ACTH-Ab~Ru(bpy) (black cap), 1 bottle, 8 mL: Monoclonal anti-ACTH antibody (mouse) labelled with ruthenium complex 0.3 mg/L; MES buffer 50 mmol/L, pH 6.2; preservative.

5.2 Calibration: Refer to ACTH CalSet leaflet.

- 5.2.1 Every Elecsys reagent set has a barcoded label containing specific information for calibration of the reagent lot. The predefined master curve is adapted to the analyser using the relevant Cal Set.
- 5.2.2 Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer) (L calibration), if more than 24 hours it will be RP calibration which must be repeated with another reagent pack otherwise of the same lot.
 - 5.2.2.1 Calibration interval may be extended based on acceptable verification of calibration by the laboratory.
- 5.2.3 Renewed calibration is recommended as follows:
 - 5.2.3.1 After 8 weeks when using the same reagent lot.
 - 5.2.3.2 After 7 days when using the same reagent kit on the analyser.
 - 5.2.3.3 As required: e.g. quality control findings outside the defined limits.
- 5.2.4 Calibration procedure: Refer to ACTH CalSet leaflet.

5.3 Quality control: Refer to PreciControl Multi-marker leaflet.

- 5.3.1 For quality control, use PreciControl Multi-marker in addition, other suitable control materials can be used.
- 5.3.2 Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.
- 5.3.3 QC procedure: Refer to PreciControl Multi-marker leaflet.

6. RESPONSIBILITIES:

- 6.1 Hormone shift on charge is responsible for, running calibration and control and samples of ACTH.
- 6.2 Hormone staff are responsible for running ACTH samples every morning.

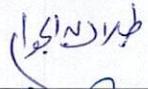
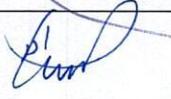
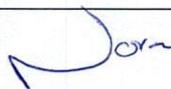
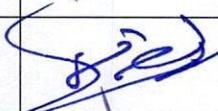
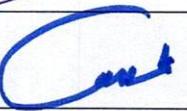
7. APPENDICES:

- 7.1 N/A

8. REFERENCES:

- 8.1 Operator's manual for Cobas e411 analyser
- 8.2 Company Leaflets of ACTH reagent, ACTH CalSet and multi-marker QC

9. APPROVALS:

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